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Studies on the Motile Function of the Fallopian Tube

Report 1. Analytic Studies on the Motile Function of the Fallopian Tube

By

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(Received for publication, March 7, 1960)

INTRODUCTION

The oviferous function of the Fallopian tube is a matter of grave concern from the view-point of reproductive physiology as being implicated in the question of the pathogenesis of sterility and ectopic pregnancy. The mechanism of egg transportation has been attributed to chemotaxis ciliary movement or muscular movement of the tube, and at present, the accepted opinion is that the process is effected mainly by muscular movement with ciliary movement playing an auxiliary part. The muscular movement of the Fallopian tube itself, however, has been sadly neglected in the past studies, so that the function of egg transportation remains still unclarified. The present author has studied the movement of the muscles of the Fallopian tube under the headings of the mode of movement, the contractility, the pace-maker activity, the fatigability and the activity of the muscles in transporting foreign bodies, examined the characteristics of the movement in different estrual periods and discussed the difference in the functions of circular and longitudinal muscles, and succeeded in obtaining all-over information on the muscular movement of the tube, as reported in the following.

MATERIALS AND METHODS

Materials: The experimental rabbits were pretreated as follows and their Fallopian tubes were examined in situ: 1) Desexualized rabbits: Mature female rabbits were bilaterally oophorectomized and subjected to examination 4 weeks after the operation: 2) Injected with estrogen: 3 weeks after similar oophorectomy, 30γ/kg/day of ovahormone benzoate suspension was injected for 7 consecutive days before examination 3) Injected with progesterone: 3 weeks after similar oophrectomy, 1 mg/kg/day was intramuscularly injected for 4 consecutive days. 4) In ovulation: Mature rabbits in estrus were injected with 5cc of urine of gravida and used for the examination 24 hours later. 5) In

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pregnancy; Rabbits 25–27 days after coition. 6) In puerperium: Rabbits 3–5 days after delivery.

Methods: The rabbits were lapartomzied at the lateral side without anaesthetization; their Fallopian tube was bared for macroscopic inspection, kept warmed with an electric heater and protected from drying with a current of warm and moist air. For examining the movement a binocular microscope was used, the isotonic or isometric contraction of the muscles was recorded with an optic lever or a mechano-electric transducer RCA 5734 and the intratubal pressure by O_2 insufflation. Somtimes, 50 cycle AC or low-frequency square current was used for applying electric stimulation.

RESULTS

1. Types of movements observed: The Fallopian tube was found subject to twitching, non-conductive segmented, conductive segmented, peristaltic, pendular, torsional and bending movements. The segmented and the peristaltic movements, considering their regular action, may be deemed to form the principal factors making up the retrograde function of the tube. I have classified the segmented movements into the types of conductive and non-conductive, the latter consisting of mere repetition of contractile movements at the same site of the tube, as shown Fig. 1a, while in the movements of the former type, the contraction propagates over a wide area, as shown in Fig. 1b. The schemata of the types of movements accompany the mechanograms of the respective movements. It is seen that the mechanogram of conductive segmented

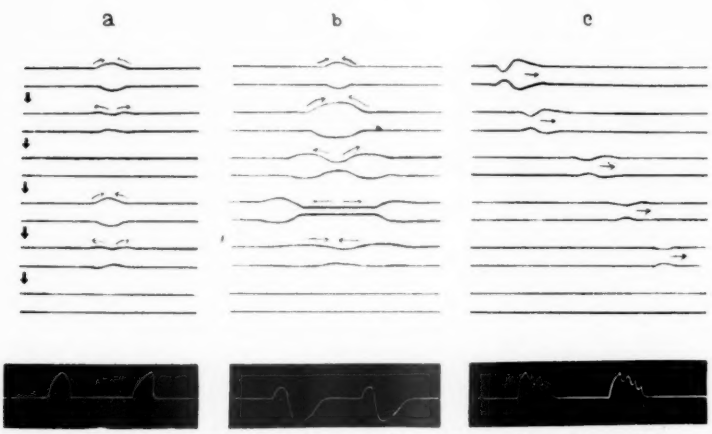

Fig. 1. Schematic and kymographic representation of the tubal contraction. a: non-conductive segmented, b: conductive segmented and c: peristaltic movement.

movement is biphasic, a long ample phase of propagation following a phase of contraction. The peristaltic movements comprise those of ampulla-uterus direction and of uterus-ampulla direction, the former prevailing overwhelmingly. In the mechanogram, such movements are recorded by serrate curves. Non-conductive segmented movements were more frequent in the ampulla, conductive segmented and peristaltic movements in the isthmus, and it was observed that in estrus and after estrogen, the movements of all types became more complicated and less in amplitude, after progesterone more simple and ample and in gestation markedly reduced in activity all over.

2. Contraction of the cricular muscles: The trend of contraction of circular muscles may be represented by the form of insufflation curve. It was

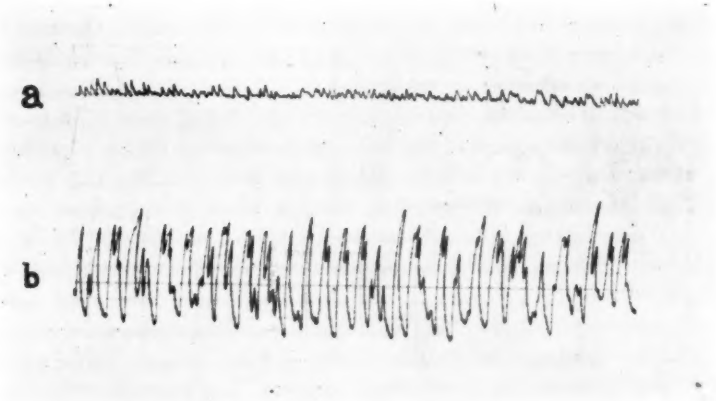

Fig. 2. Automatic circular muscular contraction.
a : estrogen and b : progesterone administered.

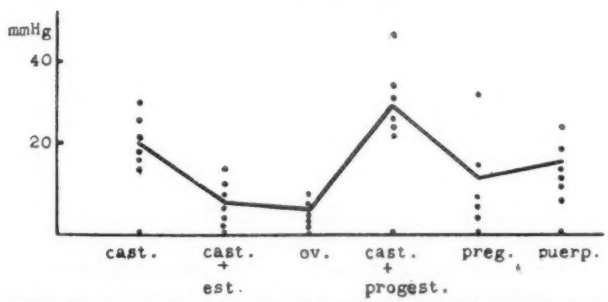

Fig. 3. Contractility of automatic circular muscular movement by O_2-insufflation. cast.: desexualized organ, cast. + est.: estrogen administered after cast., ov.: in ovulation, cast. + progest.: progesterone administered after cast., preg.: in pregnancy and puerp.: in puerperium.

found very different when estrogen is in predominance from that when progesterone is in ascendancy: as shown in Fig. 2, in the former stage, the amplitude of contraction is extremely small and its frequency is high, while after progesterone the amplitude is large and the frequency is low. Since under electric stimulation the contractile capacity of the muscles was found the larger after estrogen, it may be said that after administration of estrogen the muscles perform small contractions for their contractibility in frequent repetition and after progesterone they perform sufficiently large contractions at slow pace. Thus, the insufflation curves in each estrual period and after each hormone reflecting the automatic movements were shaped as shown in Fig. 3; these can be well interpreted if we bear such influence of the different hormones in mind.

3. Contraction of longitudinal muscles: As measured, not in the state of automatic movement but under electric stimulation, the contractile power of the longitudinal muscles at the maximum, as shown in Fig. 4, is heavily influenced by the kind of sex hormone in predominance; after estrogen the power comes up to ca. 0.5g/cm, but after desexualization or in the last stage of pregnancy it drops to about half or one-third of the value compared with estrogen administered.

4. Frequency of contraction: In the circular muscles, the frequency perceptibly rose after estrogen and lowered after progesterone. In the longitudinal muscles, the tendency was similar but not so evident. In Fig. 5 is shown the minutely frequency of contraction in segmented movements in different estrual periods.

5. Pace-maker activity: The pace-maker for the movements of the tubal muscles has not yet been histologically identified, but functionally speaking, it is quite reasonable to assume the existence of some pace-maker activity in any site wherever segmented movements occur. There are some classes of pace-makers and after progesterone only the more powerful pace-makers are in activity while the less powerful are in abeyance, after estrogen and at ovulation the

Fig. 4. Contractility of longitudinal muscular movement under electric stimulation.

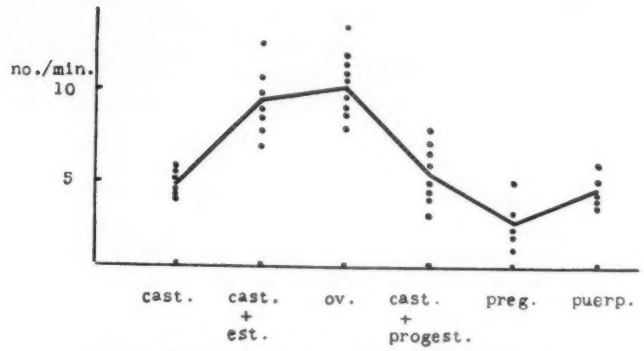

Fig. 5. Repetitious frequency of the segmentation.

pace-makers of all classes are active, but in the last stage of gestation nearly all pace-makers are in languor. In some cases, the postulation of a supreme pace-maker controlling all the pace-makers in all the portions of the tube or a coordinating action of all the pace-makers was sufficient to interpret the results.

6. Constancy: In desexualized rabbits, the tubal movements were found very stable, coming forth in monotonous rhythmical repetition. In the next place, the movements were relatively stable after estrogen and at ovulation, but some complicated overlapping of small contractions and irregular contractions of varying amplitude came to disturb the stability. After administration of progesterone, the contractions were generally regular, but a slight stimulation was apt to upset the balance of the rhythm or cause a change in the tonus, e.g. application of electric stimuli often caused irregularities in the contraction form; in this case, we may think of a blocking of the conduction of stimulation wave by the luteal hormone.

7. Fatigability: When recording of the automatic movement of the insufflation curve is continued for a long time, a phenomenon of fatigue becomes observable. The fatigue causes reduction in the amplitude and the frequency of the contractions and irregularities in the wave form. In the present study, the time elapsing before the frequency of contraction falls to one-half of the initial frequency was taken to measure the fatigability of the muscles (Fig. 6). In the last stage of gestation, the fatigability is very high, next after progesterone, not so high as generally believed after desexualization, after estrogen and at ovulation so low that apparently no fatigue was caused even after long continued recording, though the amplitude was very small from the outset.

8. Transportability: The function of egg transportation is made up synthesis of all the above-mentioned factors. When the conditions under which a foreign body was transported down the tube were experimented by introducing a drop of olive oil mixed with oil paint into the most proximal portion of the

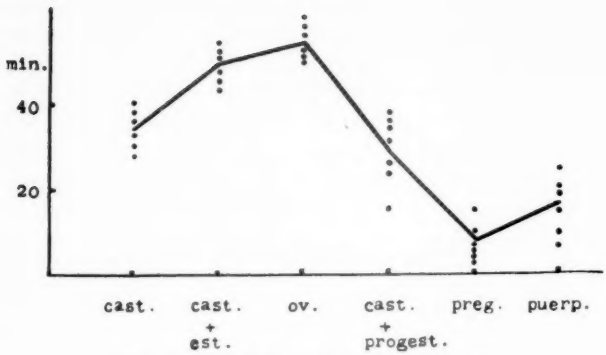

Fig. 6. Fatigability of tubal movement.

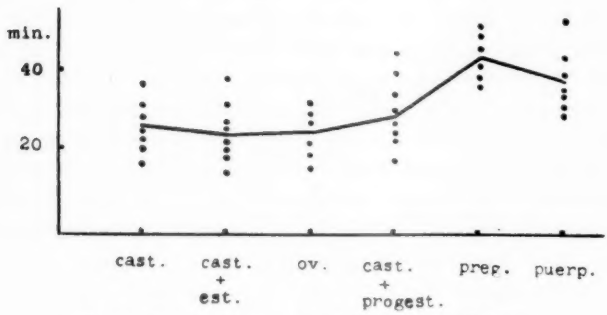

Fig. 7. Intratubal transportability of the oil-drops.

isthmus, it was found that, as shown in Fig. 7, the process went on in nearly the same tempo whether the rabbit was desexualized, injected with estrogen or progesterone or was in the period of ovulation, suggesting that though the individual factors coming into the play at the transportation may show characteristic effects varying by difference of the estrual periods, interestingly enough, the combined transporting function does not change perceptibly, except for in the stages of late pregnancy and puerperium, when the motor function of all the tubal muscles weakens all over, resulting in a lowering of the transporting function too. This transporting function was more powerful in the direction of ampulla-uterus than otherwise; this tendency was also confirmed by recording the O_2 insufflation curve.

DISCUSSION

The oviferous function of the Fallopian tube mainly depends on the coordination of the ciliary and the muscular movements in it, but Sobotta[1] was inclined to

disapprove the ciliary movement theory seeing that cilia are lacking in the distal part of the tube of guinea-pigs, mice and rats, and Tröscher[2] and Tietze[3] also were of similar opinion, considering the decrease of cilia in the ovulation stage. On the other hand, the theory of muscular movements is supported by many authors and the present author also ascribes a high significance to muscular movements in the process of egg transportation. Dyroff[4] ascertained a uterus-ampulla anti-peristaltic movement by hysterosalpingography, but according to my observations, the tubal peristaltic movements are mainly oriented ampulla-uteruswards, in agreement with what Kok[5-6], Mikulicz-Radecki[7] and Cella & Georgescu[8] have reported. In my experiments, the function of transporting foreign bodies of the tube was more facile in the direction of ampulla-uterus and Kok's experiments with colored Ringer solution and Whitelau's experiments by O_2 insufflation also showed similar results. Another type of the muscular movements consists in segmented movement, as already observed by Hirschburg[10], Mikulicz-Radecki[11] and Seckinger[12]. I classified movements of this type into those showing conductivity and those without conductivity. On the influence of sex hormones on the motor functions of the Fallopian tube, Seckinger[12], Manzi[13], Geist[14-15], Wimpfheimer[16], Rubin[17], Bernstein[18] and Binder[19] claim that estrogen acts in stimulating the functions and Cella and Beipohl[20] advocated the opposite, but it seems that the effects of estrogen and progesterone are not so simple as to be so cursorily described.

In my study, the tubal motor functions were itemized by the form of the movements, the extent and the frequency per minute of muscular contractions, the pace-maker activity, the fatigability and the transportation time, in the different periods of estrual cycle, i.e., after desexualization, desexualization plus estrogen administration, desexualization plus progesterone administration, ovulation, pregnancy and puerperium. The results gave evidence that estrogen and progesterone have respectively specific selectivity for their point of attack.

SUMMARY

The motor functions of the Fallopian tube classified into types of muscular movements and their correlation to the estrual periods and kinds of sex hormones was studied, as follows:

1. The movements of the tube were itemized into the peristaltic, the conductive segmented, the non-conductive segmented, the pendular, the bending and the torsional movements.

2. Both the circular and the longitudinal tubal muscles showed larger contractility under electric stimulation when estrogen was in predominance than when progesterone was in predominance, but the automatic contraction was smaller in the former state than in the latter.

3. The frequency of the contractions and the pace-maker activity were

higher when estrogen was in predominance than when progesterone was in ascendancy.

4. The fatigability of the muscles was lower under predominance of estrogen than under predominance of progesterone.

5. The function of transporting ova lowered in gestation and puerperium, but showed little variation in any other stage.

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Studies on the Motile Function of the Fallopian Tube

Report 2. Effect of Audio-Rectangular Pulse Wave Current on the Motile Function of the Fallopian Tube

By

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(Received for publication, March 7, 1960)

INTRODUCTION

The oviferous function of the Fallopian tube is an important matter from the view-point of reproductive physiology correlated with the question of genesis of sterility and ectopic pregnancy. As possible factors lying at the base of the process, we may enumerate chemotaxis, ciliarly movement and muscular movement, and it is accepted that the last item plays the leading role here. Therefore, the function of the tube should be adequately interpreted by elucidating its muscular movement. In the previous Report 1, the present author reported on the results of studies on the correlation of estrual periods and sex hormones with the features of the tubal movements, i.e. the muscular contraction, the form, the pace-maker activity, the fatigability and the conductivity. In succession, I conducted a study on the effect of audio-rectangular pulse-wave current (ARC) and at the same time made fundamental researches on the possibility of introducing ARC therapy into the clinical practice for treating salpingopathic cases. I had the pleasure of ascertaining that ARC therapy may be successfully adopted in treating diseases in the Fallopian tube for which only intubation, medication and surgical operation have been applied, generally with indifferent results, as reported in the following.

MATERIALS AND METHODS

Vital and excised Fallopian tubes of rabbits were used as the experimental materials.

For the experiment, the apparatus shown in Fig. 1 were designed. A is the devise for recording the contraction of the longitudinal muscles (strictly speaking, some circular muscles also come into the play); the Fallopian tube specimen mounted in the Sugi-type isolating vessel of plastics is partitioned into

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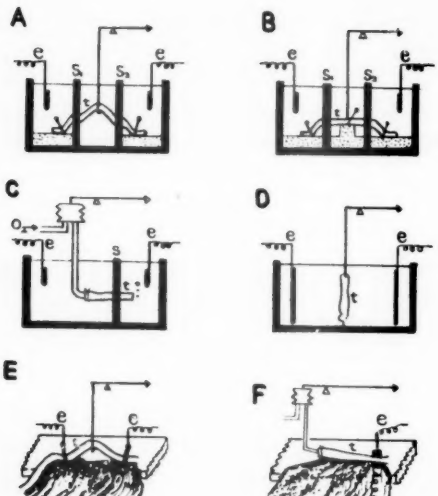

Fig. 1. Experimental apparatus.
e : Stimulating electrode. s_1 & s_2 : Separating wall. t : Fallopian tube

three sections electrically insulated from one another by the two insulating walls S_1 and S_2. The ends of the specimen were pinned down on the bottom of the vessel covered with a cork plate, and the insulation points were kept unmovable by traction of a lever. The distance between S_1 and S_2 was 1 cm and the length of the specimen between them was fixed at ca. 1.5 cm. A hook was mounted at the median point of this 1.5 cm length and the movement of the specimen at this point was transmitted to a mechanogram. B was the device for recording the contraction of the circular muscles (strictly speaking, some longitudinal muscles also come in to make up the movement) ; the tubal specimen was pinned to a cork pillar set up at the middle of the insulating box, for recording the movement in the transverse direction. C is the device for recording the insufflation curve of the tube ; a polyethylene tube was inserted from the ampullar end and O_2 gas was sent in through it at the velocity of 30 cc/min; the other end of the tube specimen was left free always for the length of 1 cm. The intratubal pressure was recorded with a Takebe's Grafax insufflation recorder. D is the apparatus mainly consisting of 2 large electrodes between which the tube specimen can be stimulated uniformly in all its parts. E and F show the process of treating vital specimens in schema. The insulating chambers were filled with Tylode's solution kept at 22–26°C for inhibiting automatic movements and aerated with O_2 or $95\%O_2+5\%CO_2$. The electrodes used for stimulation were Ag or Zn-$ZnSO_4$-agar-Ringer electrodes, which were employed in various arrangements and

combinations. The mechanograph was used to record the isotonic and the isometric contraction with an optic lever or a mechano-electric transducer RCA 5734. ARC from an electronic stimulator and 50 cycle AC were used for stimulation.

RESULTS

1. Effect on longitudinal and circular muscles: Fig. 2 shows the correlation of the insufflation pressure to the frequency of the stimulating ARC, when the voltage was kept constant at the optimal voltage, the duration of a stimulating pulse wave was kept at 1 msec. and the stimulating duration was kept at 5 sec. while the frequency is changed in the range of 1–1000 c/sec. When the frequency fell below 5 c/sec. or rose above 500c/sec., the insufflation pressure showed little change; at about 7 c/sec. a response begins to appear in the insufflation curve, rising to the maximum at 30, 50 or 100 c/sec. (in the case illustrated, at 30 c/sec.), then declining rapidly to nothing above 1/100 of that at the maximum level. A similar relation was apparent in the contraction curve of circular muscles, but the contraction curve of the longitudinal muscles was somewhat different, the frequency of maximum effect being scattered more widely, often up to 900 c/sec., though in most cases the range between 50 and 300c/sec. was the normal. In the correlation between the threshold voltage and frequency of the stimulating current too, the longitudinal and the circular muscles showed some dissimilarity, the latter being found to respond effectively to ARC of a lower range of frequency than the former. Thus, it was revealed that the longitudinal and the circular muscles of the Fallopian tube have respectively different electrophysiological characteristics, and that the insufflation of the tube is almost entirely due to the contraction of circular muscles. The other three factors, voltage, duration of the

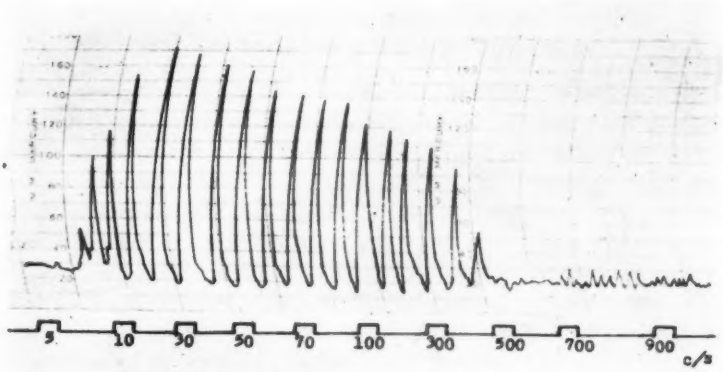

Fig. 2. Effect of ARC frequency on the circular muscular contractility. Signals show frequency in c/sec.

Fig. 3. Effect of ARC voltage on the longitudinal muscular contractility. Abscissa : Voltage in V. and ordinate : Contractility in percentage (max. contraction is decided on 100%).

pulses and the duration of the applied stimulation in the experiment showed no specific difference in the response of longitudinal and circular muscles. When the amplitude of a certain stimulated contraction, by percentagment compared with the maximum contractibility of the tube which was decided to be 100%, is applied as the measure of the effectiveness of stimulation, the results under varying voltage and duration of a pulse of applied stimulation were as follows. The

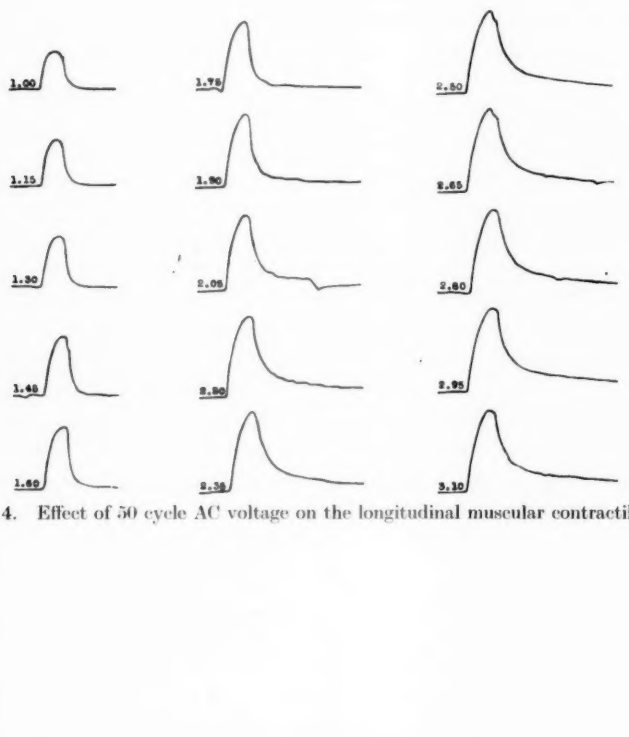

Fig. 4. Effect of 50 cycle AC voltage on the longitudinal muscular contractility.

effect of changing the voltage was unequal under different frequency, as shown in Fig. 3, for while at the duration of pulses of 1 msec. and the duration of the stimulation of 5 sec., the response reached the maximum at 5V or so in the range of frequency of 100–200 c/sec., when the frequency is too low (5–10 c/sec.) or too high (500–900 c/sec.) usually no raise of the voltage can effect maximum contraction. As in Fig. 3, however, many individual results of measurements under stimulation of the longitudinal muscles are illustrated, some exceptional individual differences are observable, e.g. in one case, the maximum response is seen in the range of frequency of 600–900 c/sec.. In the electric field of a 50 cycle AC, the current density of around 2 V/cm seemed to be most effective in stimulating longitudinal muscles, considering the change in the wave form of contraction (Fig. 4); a lower voltage was insufficient in causing maximum contraction, while a higher voltage would cause delay in the recovery of the contracted muscle.

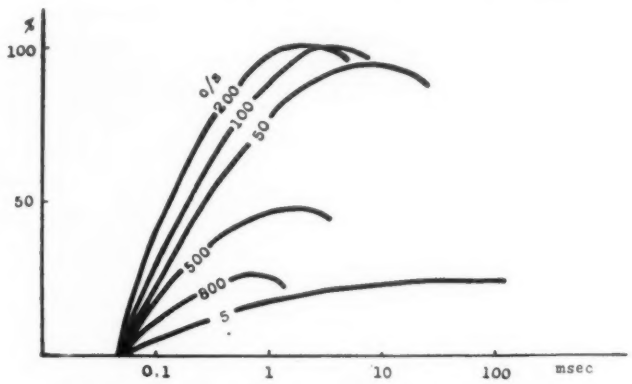

Fig. 5. Effect of ARC pulse duration on the longitudinal muscular contractility. Abscissa: Pulse duration in msec.

Under constant voltage of 5V and stimulating period of 5 sec., as exemplified in Fig. 5, a longer duration of the electric pulse caused stronger contraction of the longitudinal muscles, the maximum contraction being realized when the pulse duration was slightly beneath that compatible with the frequency of the applied ARC. When the frequency was outside the effective range, no prolongation of the pulse duration could bring about maximum contraction, while within the range of 0.05–0.01 msec. of duration no variation of the frequency could effect realizing the maximum in muscle contraction either.

When the voltage was kept fixed at 8 V and the pulse duration at 1 msec., the contraction of the circular muscles under varying duration of the applied stimulation was as shwon in Fig. 6. When the stimulation was prolonged, the contraction is seen to amplify, but the mode of contraction shows that an over-

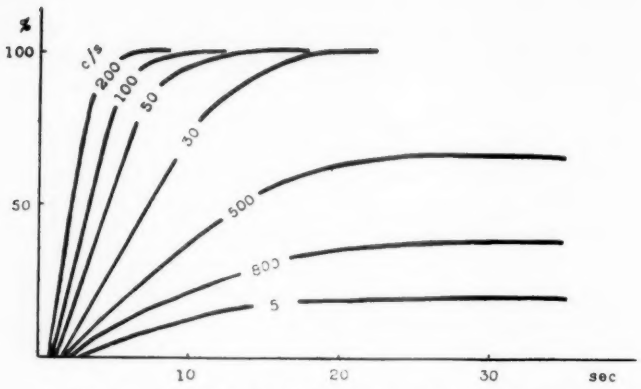

Fig. 6. Effect of ARC polarization on the circular muscular contractility. Abscissa: Polarization in sec.

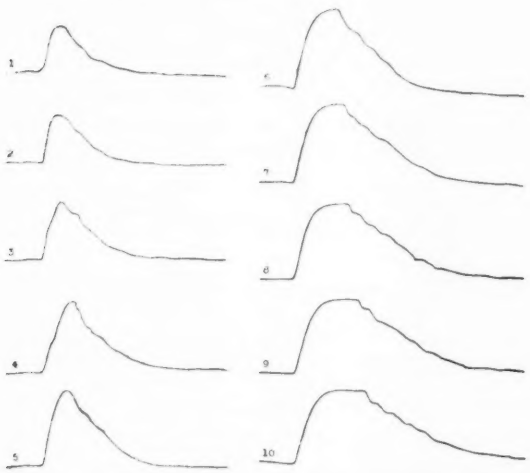

Fig. 7. 50 cycle AC polarization on the longitudinal muscular contractility. The numerals show polarization duration in sec.

prolongation of the stimulation results in forced abnormal contraction. As shown in Fig. 7, under stimulation with 50 cycle AC, the longitudinal muscles lose the capacity of recovery after overprolonged stimulation. The optimum duration of stimulation was found to range between 3 and 5 sec., as revealed by contemplation of the curves.

2. Effect on the type of movement: Movements of the Fallopian tube of the segmented type, conductive as well as non-conductive, and peristaltic type

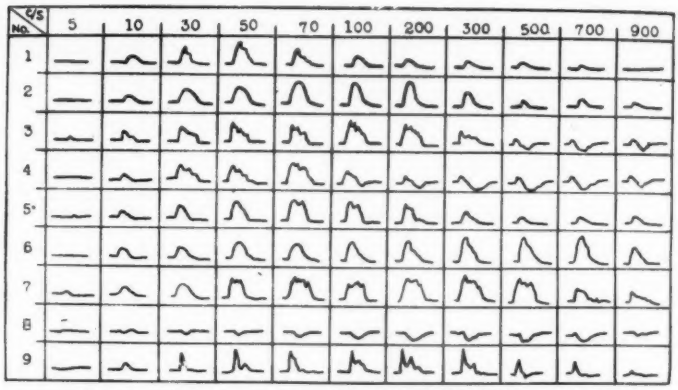

Fig. 8. ARC frequency on the mode of the Fallopian tube.

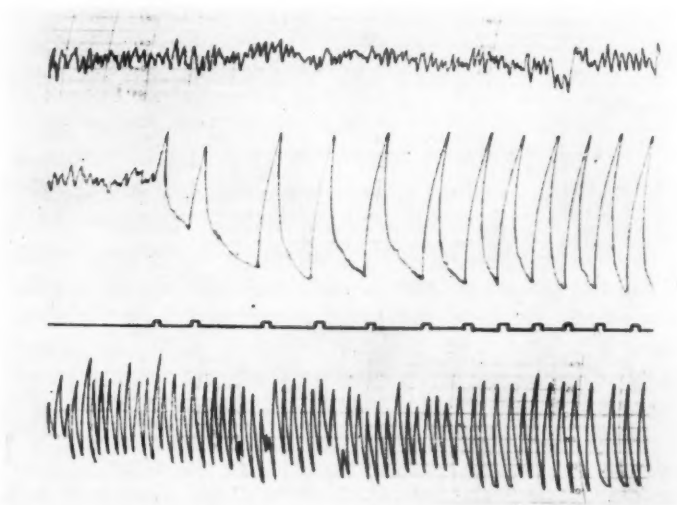

Fig. 9. Spasmolytic action of ARC on motile function of the Fallopian tube. Upper shows spastic tubal contraction, middle ARC polarized and lower success in spasmolysis.

could be induced by ARC stimulation. The curves and the conductivity of the movements induced depended on the moving site and the stimulated point of the tube and the mode of applying the tonus, as well as the estrual periods and the individual differences, but as shown in Fig. 8, we might summarize that the form of the movement is generally correlated with the frequency of the stimulating ARC in a definite way; for peristaltic movements and conductive segmented

movements showed selective response to ARC of 30–300 c/sec. and 100–900 c/sec. frequencies respectively. At 5–20 c/sec., only non-conductive segmented movements and twitches could be induced, but movements of these types did always come forth, whatever the frequency of the current is.

3. Effect on the motor function: When a contracted tubal muscle in a state of convulsion is polarized with ARC of 30–50 c/sec. frequency, the tonus is slowly slackened, the amplitude is gradually widened and the rhythm recovers regularity from the original irregularity (Fig. 9). Under stimulation with 50 cycle AC too the result was similar, but only less prominent. ARC polarization also showed reactivating effect; an excised specimen fatigued in a salt solution could be made to recover from fatigue with ARC of 30–500 c/sec. frequencies. Such a reactivating effect can be inferred from the fact that the resuscitation is expedited by ARC polarization in the case of revitalizing excised specimens in nutritive solution. Anodal polarization seemed preferable in effecting such activation, probably because application of anodic potential enhances the permeability of a membrane and thus contributes to the improvement of the conductivity and enlarges the amplitude. Such spasmolytic and activating effects were manifest in the cases of both the longitudinal and the circular salpingian muscles.

DISCUSSION

The leading opinions on the oviferous mechanism support either the ciliary motion theory or the muscular movement theory. Grosser[1] and Kneer[2] observed that foreign bodies in the Fallopian tube is carried on by movement of cilia, but Sobotta[3] pointed out that in guinea-pigs, mice and rats the distal portion of the tube is devoid of cilia and Tröscher[4] and Tietze[5] observed that cilia decrease in number in the period of ovulation, so that the ciliary motion theory is tainted with some hardly reconcilable defects.

On the other hand, the muscular movement theory has obtained support by many authors; Kok[6], Gogoberidse[7], Mikulicz-Radecki[8] and Doyle[9] conducted experiments on the mode of movement and oviferous process in human and some animal salpinx. Cella[10], Manzi[11], Geist[12–13], Wimpfheimer[14], Bernstein[15] and Binder[16] studied the correlation between the muscular movement and sex hormones, and Wimpfheimer,[17] Rubin[18–19], Marbach[20] and Davids[21] perfected methods of gastubation in their attempt to grasp the salpingian muscular movements from the clinical stand-point. The present author has previously reported in Report 1 on the study on such movements as analysed from the angle of muscular contraction, form of movement, pace-maker activity, fatigability and transporting ability and in correlation with the different estrual periods and sex hormones. Csapo[22] has made electrophysiological experiments on uterine muscles, but no data of such studies on the Fallopian tube has ever been published. I

have elucidated by experiments that the longitudinal tubal muscles show electrophysiological characteristics different from those of the circular tubal muscles — an interesting discovery, I believe. The findings that ARC has the effects of spasmolysis and activation on salpingian muscles indicate that ARC therapy for salpingopathies is at least in principle as possible as for diseases of skeletal muscles and nerves. The effective action of such a therapy will be based on Suzuki's third effect[23] of the polarizing electrodes in raising the excitability at the anode and lowering it at the cathode. I am now devising an apparatus for applying ARC to the vital human Fallopian tube and will report the clinical results obtained with it in the future.

SUMMARY

The effects of audio-rectangular pulse wave current (ARC) and 50 cycle alternating current on the Fallopian tube of rabbits were studied and the following results were obtained:

1. The longitudinal and the circular salpingian muscles showed different responses to ARC, the optimum frequency being 50–900 c/sec. for the former and 30–100 c/sec. for the latter.

2. The correlation of the frequency, the voltage and the duration of pulses and the duration of applied stimulation with ARC to the intensity of muscular contraction was studied also.

3. ARC showed selective effect on different types of the muscular movements.

4. ARC was found to have spasmolytic and activating effects when applied to the salpingian muscles.

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Carotid Sinus Reflex Acting upon Shivering

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Shivering is regarded as an important process for chemical regulation of body temperature. Attempts have been made by many investigators to find out the cause of shivering in the function of the thermoregulatory center. On the other hand, Takagi[1] reported in his study of skin pressure reflexes that shivering was inhibited by pressure applied to the skin and augmented by touch. These facts suggest that shivering may be controlled by peripheral nerves.

As a part of studies upon the reflex control of shivering, investigation was made on action from the carotid sinus.

METHOD

Rabbits weighing about 2.5 kg were employed. Twenty-five per cent urethane solution was injected subcutaneously in a dose of 1.5 g per kg of body weight. Inducing the myogram from adductors of the lower limb with a co-axial needle electrode, shivering was recorded by means of an electromagnetic oscillograph. Blood pressure was recorded from the femoral artery and the central or peripheral end of the common carotid artery by a tambour provided with a minute mirror through Ludwig's mercury sphygmomanometer, together with the myogram.

Increase or decrease in the pressure of carotid sinus was brought about by clamping the common carotid artery with a clip or loosening it. In some experiments the carotid sinus was isolated from the vascular system, its innervation being preserved. The pressure in it was raised by infusion with Ringer solution. Ten mg of heparin per kg of body weight was sometimes used for preventing the coagulation of blood.

RESULTS

The animals were fixed in supine position under the cool environment (5~20°C). Since shivering was weak at onset; experiments were started when shivering became intense.

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1. Experiments of raising and lowering the carotid sinus pressure

When the blood pressure in the carotid sinus was lowered by pinching the common carotid artery with clips, the general blood pressure rose from about 115 mmHg to 120 mm Hg and shivering was inhibited simultaneously, but the rate of inhibition was not so remarkable. When the depressor nerves had been previously divided, this effect became more marked. In this case increase of the general blood pressure was greater, such as from 120 mm Hg to 130 mmHg. When the carotid sinus pressure was raised by loosening the clips, shivering was intensified transitorily. At that time the general blood pressure fell rapidly, and about 10 seconds later its normal level was restored. With the return of blood pressure to the normal, shivering resumed its normal condition (Fig. 1).

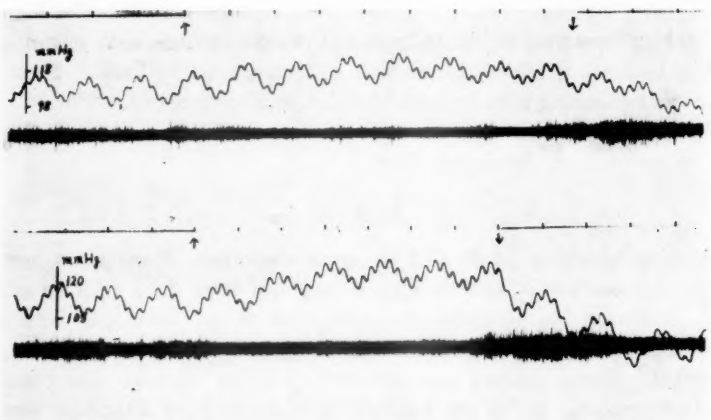

Fig. 1. Effect of lowering the carotid sinus pressure on shivering.
1) Carotid sinus pressure was lowered by fastening the common carotid artery with a clip (↑—↓).
2) After cutting depressor nerves, carotid sinus pressure was lowered in the same way.
Blood pressure was recorded from the left common carotid artery.
Rabbit; ♀, 3 kg. Urethane; 3.75 g. Heparin; 25 mg. R.T.; 10°C. Time; 1 sec.

With isolated carotid sinus, its innervation being intact, the augmentation of shivering could be maintained so long as its internal pressure was raised by infusion of Ringer solution. With lowering this pressure shivering was inhibited (Fig. 2).

After cutting the depressor and vagal nerves at the neck, the fall of the carotid sinus pressure caused in most cases a complete inhibition of shivering for 45 minutes, and the general blood pressure rose from 120 mm Hg to 175 mm Hg. At that time, a rise in the carotid sinus pressure induced strong shivering, and the

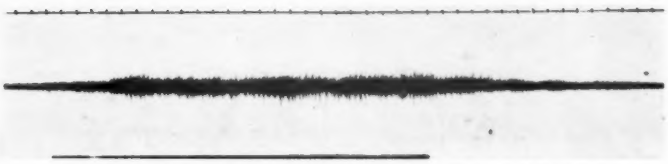

Fig. 2. Effect of raising carotid sinus pressure on shivering.
The pressure within the isolated carotid sinuses with intact innervation was raised from 0 mm Hg to 210 mm Hg by infusing Ringer solution.
Rabbit; ♂, 2.4 kg. Urethane; 2.8 g. R.T.; 12°C. Time; 0.5 sec.

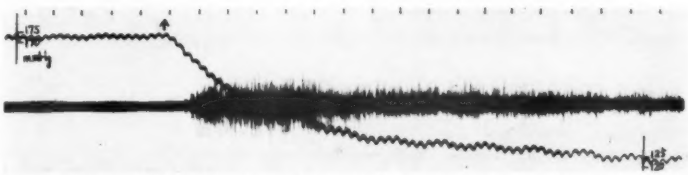

Fig. 3. Effect of raising the carotid sinus pressure on shivering.
Vagal, depressor and sympathetic nerves were cut previously at the neck. By fastening common carotid arteries with clips shivering was inhibited completely for about 45 minutes. The clips were removed at ↑, and intense shivering set in while general blood pressure fell down from 175 mm Hg to 125 mm Hg.
General blood pressure was recorded from femoral artery.
Rabbit; ♀, 3.5 kg. Urethane; 4.5 g. Heparin; 25 mg.
R.T.; 11°C. Time; 1 sec.

general blood pressure fell from 175 mm Hg to 125 mm Hg. Shivering became weak until its original condition was restored with the return of blood pressure to the normal level (Fig. 3).

2. *The effect of change in blood pressure due to cutting the depressor and vagal nerves on shivering*

It was found occasionally that on cutting the depressor or vagal nerves at the neck shivering was inhibited and suspended for a while. The relationship between the change of blood pressure and shivering was therefore observed.

By cutting the depressor nerves, the general blood pressure at 95 mm Hg began to fall gradually in 2 seconds and reached 60 mm Hg about 10 seconds later, then it began to rise after about 15 seconds and restored its original level in the following 60 seconds. Shivering vanished with the fall of blood pressure below 70 mm Hg. As the blood pressure restored about 70 mm Hg, shivering appeared again (Fig. 4).

In the case of cutting the vagal nerves, the results were almost the same, the height of 70 mm Hg being the turning point in blood pressure for disappearance and restoration of shivering. Generally shivering was synchronous with

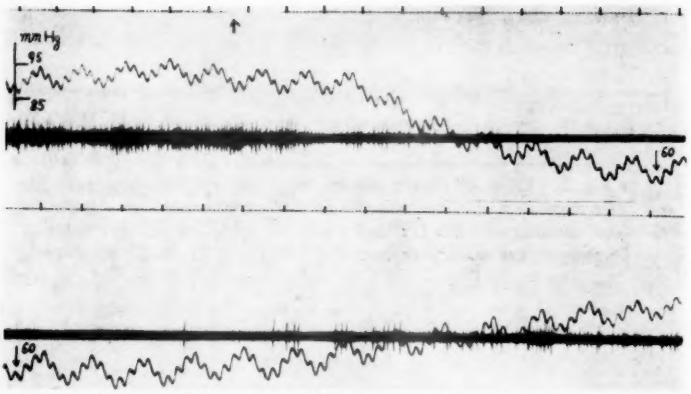

Fig. 4. Changes of shivering and blood pressure by cutting depressor nerves.
Arrow indicates cutting of right depressor nerve at the neck.
Blood pressure was recorded from right femoral artery.
Rabbit; ♀, 3.2 kg. Urethane; 4.5 g. Heparin; 23 mg. R.T.; 7°C. Time; 1 sec.

respiratory fluctuation. The synchronism was more eminent with rise in blood pressure.

3. Shivering at lowering the blood pressure by venesection

From the above experiment, it may be considered that shivering has relationship with the blood pressure. Experiments with venesection were thus performed.

When the blood pressure was lowered to 70 mm Hg or below by venesection from femoral artery, shivering vanished out. The subsequent rise of blood pressure by blood transfusion above 70 mm Hg resulted in reemergence of shivering. This relationship took place reversely. Generally the higher the blood pressure the more intensive shivering became.

4. Relation between the blood pressure and shivering by injection of adrenalin

When the rabbit was shivering, 0.5～1.0 cc of 10^{-4}～10^{-5} adrenalin was injected into the auricular vein. Shivering was inhibited immediately and suspended completely for about 15 minutes, then gradually shivering set in. However, after cutting both vagi in advance, the effect of adrenalin was entirely different from the case of intact vagi. By the injection of adrenalin shivering became intense with the rise of blood pressure and weakened gradually according to the lowering the blood pressure. Then shivering was suspended with an extraordinary fall of blood pressure. As the blood pressure was restored to the normal level, shivering regained its original condition.

Shivering which was intensified by adrenalin injection was inhibited completely by the fall of carotid sinus pressure caused by fastening the common carotid arteries with clips and was recovered on release of the clips. From

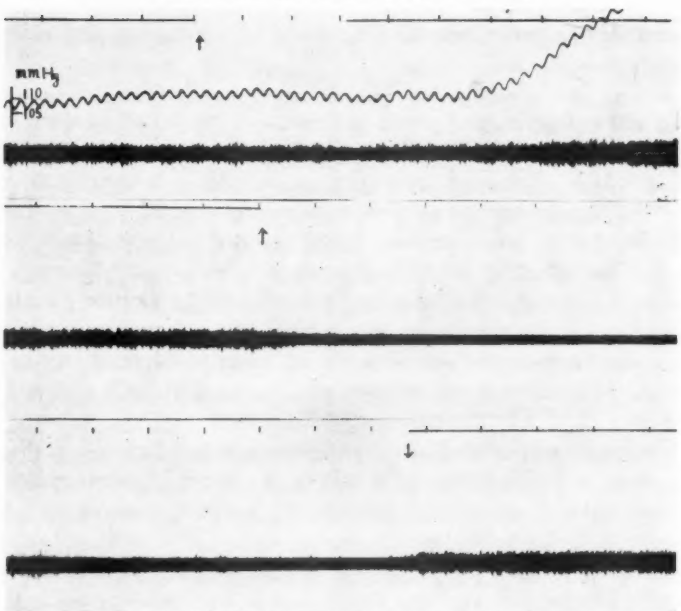

Fig. 5. Effect of adrenalin injection on shivering.

1) 0.5 cc of 10^{-4} adrenalin was injected into the auricular vein (↑) after cutting vagal, sympathetic and depressor nerves at the neck.

2)–3) (Continued 1) Fastening common carotid arteries (↑) inhibited shivering and loosening them (↓) accerelated shivering.

Blood pressure was recorded from right femoral artery and its maximal height attained to 190 mm Hg.

Rabbit; ♀, 2.7 kg. Urethane; 4 g. Heparin; 25 mg. R.T.; 9°C. Time; 1 sec.

this experiment, the augmentation of shivering by injection of adrenalin was considered to be due to the reflex action from the carotid sinus elicited by the rise of blood pressure (Fig. 5).

Both common carotid arteries were occluded in advance, and the same amount of adrenalin was injected. It was found that shivering was produced in about 20 seconds after injection and increased further gradually. This apparently paradoxical result could be explained by measuring the carotid sinus pressure. The pressure was 30∼40 mm Hg before adrenalin was injected. With the adrenalin injection the pressure began to increase in about 10 seconds and attained the maximum height of 70∼80 mm Hg about 20 seconds later. Shivering set in 5 to 10 seconds after the rise of the blood pressure, and reached their peak when the carotid sinus pressure attained the maximum value. It can be inferred that even though the blood stream in the common carotid artery was interrupted, the pressure in the carotid sinus was raised through the vertebral

artery and shivering was induced.

DISCUSSION

The above experimental results indicate that the blood pressure strongly affects shivering. Exactly the rise of blood pressure intensifies shivering while the fall inhibits it. Moreover, the experiment on raising or lowering the internal pressure of the isolated carotid sinus urges us to infer that shivering may originate from the action of peripheral nerves. Koch[2] reported the inhibition of voluntary movements in dog by increasing the pressure in carotid sinus. Tournade *et al.*[3] demonstrated in the dog that shivering was inhibited by electric stimulation of the central end of the sinus nerve. In rabbits, the authors found that the rise in carotid sinus pressure did not cause the inhibition of shivering but the excitation instead. The reason for the discrepancy of our results from Koch's and other's results is possibly due to the difference of the animal species employed.

Kishi[4] observed in dogs that shivering was inhibited by lowering the carotid sinus pressure, and he explained this fact as the failure of center caused by an impediment of blood circulation. The authors, however, consider the inhibition to be due to the reflex action from the carotid sinus. After cutting the vagal and depressor nerves on both sides, clamping the common carotid arteries with clips suppressed shivering for more than 30 minutes in most cases ; even in this case loosening the clips provoked shivering immediately. These facts indicate that this inhibition of shivering was not due to the central failure, because it is difficult to consider that the paralysis of the center lasting for 30 minutes can be restored without delay by reopening the blood flow.

It is well known that the aortic arc has the same nature as the carotid sinus as pressoreceptor in the vascular system. Pinching the common carotid artery lowers the carotid sinus pressure and raises the blood pressure in the aortic arc, namely it gives contradictory effects to these two regions. In this case the reflex action due to the pressoreceptors in these two regions should be mutually compensative, so that the lack of one should remove its compensation and push forward the other's effect. As Fig. 1 shows, the inhibitory effect from the carotid sinus on shivering became remarkable by cutting depressor nerves. This result denotes that also on shivering the pressoreceptor in the aortic arc acts in the same manner as the pressoreceptor in the carotid sinus.

There has been a number of literatures upon the effects of adrenalin on shivering. Hall *et al.*[5] reported on their experiment employing cats that the epinephrine caused a transient acceleration of shivering followed by a long-lasting inhibition. They attributed the initial augmentation of shivering to the increase of excitability in the skeletal muscle and not to the reflex action from the carotid sinus resultant from the rise of blood pressure by adrenalin, and the subsequent inhibition to the central failure. Through the experiments on dogs,

Tournade *et al.*[6] proved the inhibition of shivering by epinephrine even after cutting the sinus and depressor nerves. Kishi[4], however, with the dog demonstrated the acceleration of shivering by the adrenalin injection.

In our experiment, when vagal nerves had been cut beforehand, the injection of adrenalin caused a considerable augmentation of shivering with the rise of blood pressure simultaneously. Moreover, this enhancement of shivering was maintained during the rise of blood pressure. After both common carotid arteries had been occluded by clamping, the injection of adrenalin did not bring about the excitation of shivering for a time in spite of an extraordinary rise of general blood pressure. When the carotid sinus pressure rose over 70 mm Hg through the intervention of vertebral arteries, shivering set in and graudally became intense. These results gave us enough evidence to conclude that the augmentation of shivering by adrenalin should be caused reflexly from the carotid sinuses, and is neither of the central origin nor of the direct action upon the muscles. Adrenalin may act to change the excitability of the center and the muscle, but on shivering its effect is considered to be no more than additional one.

In contrast to the above results, injection of adrenalin suppressed shivering almost completely when the vagal nerves were intact. On the other hand, in animals with the intact vagi a fall of pressure in the carotid sinus caused a moderate inhibition of shivering. When both vagal nerves were previously divided this fall in pressure may suppress shivering completely. Cort *et al.*[7] asserted that in the pig the cooled air introduced into the trachea evoked shivering and shivering was partly accelerated through the intervention of the vagal nerve from the thermoreceptor in the lung. From the above results the authors interpreted that there are two kinds of afferent nerve fibers in the vagus, one is facilitatory, the other inhibitory. No decisive conclusion can yet be drawn.

SUMMARY

1. Increasing the carotid sinus pressure enhances shivering, while decreasing inhibits it.

2. In general, the rising of blood pressure causes an augmentation of shivering as the result of the reflex action from the carotid sinus and aortic arc. Shivering does not set in below the general blood pressure about 70 mm Hg.

3. With vagal nerves intact, shivering is inhibited by adrenalin injection, while after cutting vagal nerves the injection of adrenalin accelerates shivering.

4. It may be assumed that both the excitatory and the inhibitory afferent fibers on shivering exist in the vagal nerve.

5. The intensity of shivering changes synchronously with respiration in most cases, seemingly due to the respiratory fluctuation of blood pressure.

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Relation of Blood Pressure to Shivering

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In the previous paper[1] the authors reported that shivering was accelerated with the rising of blood pressure, being inhibited with the falling. These effects of the blood pressure on shivering were proved to be the reflex action from the carotid sinus and aortic arc.

Shivering is by no means observed in poikilothermic animals, but only in homoiothermic animals. So it is presumed that shivering is closely related to the temperature regulation. Therefore we should search for the relation between the body temperature and shivering. It is also well known that the hypothermia can be easily induced by fixation[2]. The authors have attempted to investigate the following problems;

1. the body temperature of animals which were fixed on a board, 2. the body temperature at the onset of shivering, and 3. the relation of body temperature and blood pressure at the onset of shivering.

METHOD

Rabbits weighing about 2.5 kg were anesthetized by 25% urethane solution (Wako, 1st class). The body temperature was measured every 10 minutes 6 cm deep in the rectum. The blood pressure in the femoral artery was measured by means of Ludwig's mercury sphygmomanometer.

RESULTS

1. Change of body temperature under fixation

The fixation caused a fall of the body temperature of a non-anesthetized rabbit about 2°C in 30 minutes, and the body temperature remained at this level so stable as not to show any more remarkable change for several hours. This fall of body temperature traced a fixed course whenever the rabbits were in good condition, though a little daily variations were observed. In figure 1 an average falling process of body temperature calculated on 15 rabbits was illustrated along with the 95% critical region. However, when rabbits were perceived to

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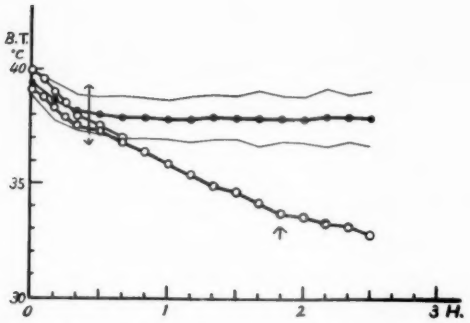

Fig. 1. Average falling process of body temperature on 15 rabbits fixed in supine position, along with the 95% critical region.
○—○—○ : Falling process of body temperature of rabbit in bad condition.
Arrows : Onset of shivering.

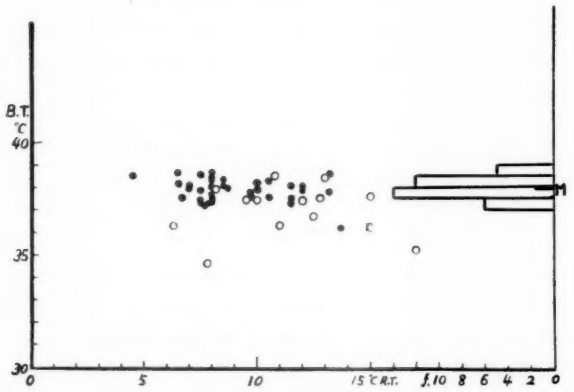

Fig. 2. Correlation between the room temperature and the critical body temperature for shivering.
● : Non-anesthetized rabbits. ○ : Anesthetized rabbits.
Histogram of critical body temperature for shivering was calculated only on non-anesthetized rabbits. M : Mean.

be in abnormal condition, i.e. lack of appetite or having shortness of breeding, the body temperature fell rapidly and fluctuated. But such a rabbit regained the normality when restored to normal physical condition.

In normal rabbits shivering was found to set in with relatively high body temperature, having no correlation with the room temperature ranging from 5°C to 20°C. The body temperature at which shivering set in, i.e. the critical

body temperature for shivering, was 37.9°C on an average (Fig. 2). When the condition of animals was bad, the rabbit began to shiver with the low body temperature and shivering itself was weak. Either the fall of body temperature or the critical body temperature for shivering was greatly variable according to animal's condition. Therefore, in order to standardize the condition of animals, the authors selected such rabbits that the falling process of body temperature was involved within the bound of 95% critical region as shown in Fig. 1, and employed them in the following experiments.

To investigate the effect of anesthetization on the above phenomenon, the authors have administered the various doses of narcotics to the rabbits and fixed them after 1 hour. By light anesthetization the fall of body temperature was not remarkable before the rabbits were fixed. In deeply anesthetized rabbits, the body temperature fell gradually (about 2°C in 1 hr.) and the fixation further accelerated its fall. The falling rate increased and the critical body temperature for shivering lowered in proportion to the amount of the narcotics (Fig. 3).

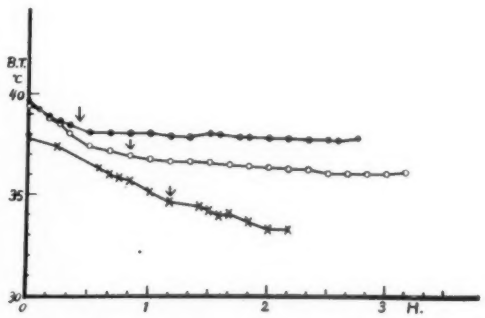

Fig. 3. Effect of anesthetization on falling process of body temperature by fixation.
●—●—● ; Non-anesthetized rabbit, ♂, 2.55 kg.
○—○—○ ; Urethane 1.5 g/kg.
×—×—× : Urethane 2.5 g/kg.
Arrows : Onset of shivering.

2. Correlation between the blood pressure and the critical body temperature for shivering

By applying the various doses of narcotics, the authors succeeded in causing shivering to set in on various body temperature and sought the relation between the blood pressure and the critical body temperature for shivering. The result of these experiments which were carried out on 14 rabbits is shown in Fig. 4.

The products of the value of blood pressure at onset of shivering and the falling degree of body temperature were set upon one line. Let the blood pressure

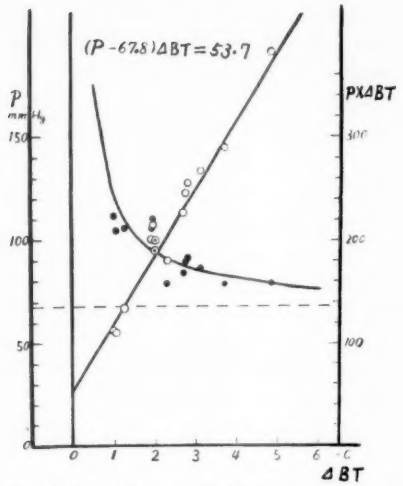

Fig. 4. Correlation between the blood pressure and the critical body temperature for shivering on male rabbits.
P: Blood pressure at the onset of shivering.
ΔBT: Falling degree of body temperature from the normal.

Fig. 5. Respiratory fluctuation of shivering and blood pressure.
Rabbit; ♂, 3.2 kg. Urethane; 3 g. Heparin; 25 mg. R.T.; 10.5°C.
Time: 1 sec. Blood pressure was recorded from left femoral artery.

and the falling degree from the normal body temperature be P and ΔBT respectively, the following correlation formula was obtained with the confidence of 99%.

$$(P-67.8)\,\Delta BT = 53.7$$

As this curve has two asymptotes of P=67.8 and ΔBT=0, it may be considered that shivering will not set in unless the blood pressure is infinitely high under the normal body temperature. This is concordant with the fact that shivering is never observed under the normal body temperature. If the blood pressure falls lower than 67.8 mm Hg, shivering will not occur in any body

temperature. This also coincides with the fact that shivering was completely inhibited by blood pressure lower than 70 mm Hg as described in the previous paper.

Shivering was often synchronous clearly with respiratory fluctuation (Fig. 5). And this synchronism was marked when the blood pressure hovered around 70 mm Hg. The periodicity of shivering is assumed to be caused by fluctuation of blood pressure around the asymptote of P=67.8. It is considered that the respiratory fluctuation on intensity of shivering is partly due to the change of blood pressure.

DISCUSSION

It has long been known as a restraint hypothermia that the animal's body temperature falls with fixation. von Bormann *et al.*[2] attributed the restraint hypothermia to increase of body surface and to a great heat loss. In their extensive experiments on the effects of skin pressure upon the various functions, Takagi[3] and Hasegawa *et al.*[4] asserted that the pressure on the skin imposed by tight fixation might reflexly affect the excitability of the thermoregulatory center. Grant[5] and Bartlett *et al.*[6] regarded the hypothermia induced by restraint as a result of emotional excitation under unusual environment.

Our experiments proved the following fact: When anesthetized rabbits were left without fixation, the exposed body surface was large enough to comply with Bormann's insistence, but the body temperature fell less than what we expected, and once fixed, the body temperature began to fall dynamically. We can not explain the rapid fall of body temperature by fixation with the increase of body surface only. Grant's concept is also not acceptable, based on the fact that fixation of the anesthetized animals which did not respond with emotional change to any stimulus still caused a remarkable fall of body temperature. Therefore it is reasonable to consider that the restraint hypothermia is due to the skin pressure reflex, resulting in the inhibition of the thermoregulatory center, as Takagi presented.

On the other hand, Nielsen[7] and von Euler[8] assumed the existence of body thermostat for temperature regulation and that the body thermostat of the center would be "set" at different levels in accordance with the change of room temperature. In the rabbits selected by suitable method, we found that the body temperature which began to fall immediately by fixation reached to a constant level in 30~40 minutes and maintained this level for 2~3 hours. Furthermore, the falling rate, this constant level and the critical body temperature for shivering showed little variation and no correlation to the room temperature ranging from 5°C to 20°C. This results lead us to the following consideration. Under the restrained condition the thermoregulatory center may keep an ability of controlling the body temperature, maintaining the heat ballance, so far as the environmental

temperature does not exceed a certain limit. The hypothermia by fixation seems to be caused by shifting the controlling level to a lower one, as Nielsen[7] asserted, but we have no particulars yet.

It is well known that the vasomotor center takes part in the peripheral vasoconstriction at the hypothermia. As we have discussed in the previous paper, the increase of the blood pressure augmented shivering and the decrease inhibited it. So we can expect that a fixed correlation should exist between the blood pressure and the critical body temperature for shivering. The authors investigated the correlation of the critical body temperature for shivering and the blood pressure, and obtained the following formula.

$$(P-a)\,\Delta BT = b$$

Herein P is blood pressure, ΔBT fall of body temperature, a, b, the constants. In our experiment a=67.8, b=53.7.

This correlation formula expresses an interdependence of the vasomotor and thermoregulatory center. It is interesting to know that such a simple relation exists between the centers in the subcortical area.

SUMMARY

1) When rabbits are fixed on animal board, their body temperature falls to a fixed level and shivering sets in. The fall of body temperature traces a fixed course and the critical body temperature for shivering shows little variation whenever the animals are non-anesthetized and in good condition.

2) When anesthetized rabbits are fixed, the fall of body temperature increases and the critical body temperature for shivering becomes lower in proportion to the amount of narcotics. Room temperature ranging from 5°C to 20°C did not have any marked effect on these phenomena in both anesthetized and non-anesthetized rabbits.

3) At onset of shivering, the following correlation formula consists between the blood pressure and the body temperature;

$$(P-a)\,\Delta BT = b$$

Both "a" and "b" are constants, "P" representing the value of blood pressure, "ΔBT" expressing the value of falling degree below the normal body temperature.

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A Correlation Between Nuclear Diameters of Oral Epithelial Squamous Cells and Blood Vitamin B_{12} Levels of Children with Nutritional Dystrophy

Studies on Nutrition of Children at Hirosaki Area (83rd Report)

By

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The characteristic cellular and nuclear changes in epithelial cells in pernicious anemia were reported for the first time by Graham *et al.*[1] in 1954. According to these authors the squamous epithelial cells, gastric columnar cells and histiocytes which were found in gastric washings, showed the changes characterized by the following three findings ; 1) an increase in size of both nucleus and cytoplasm, 2) multinucleation, and 3) a change in chromatin distribution. These nuclear and cellular changes were said to disappear rapidly after vitamin B_{12} therapy. From these findings they were of opinion that vitamin B_{12}, which was necessary for normal maturation of red blood cells, appeared to be essential for the maturation of other types of cells, such as squamous epithelial cells, gastric columnar cells and histiocytes.

In 1958 Boen[2] examined the changes of squamous epithelial cells and of their nuclei in pernicious anemia, by using mouth washings instead of gastric washings such as adopted by Graham *et al.*[1], and found the similar changes of cytoplasm and of nucleus of squamous epithelial cells obtained by mouth washings as those observed in materials obtained by gastric washing by Graham *et al.*[1]. According to Boen[2] vitamin B_{12} deficiency caused a disease involving the changes of different types of body cells. Besides disorders of hematopoietic and nervous system there were, according to this author, also characteristic changes of squamous epithelial cells of the mouth : the squamous epithelial cells, which were obtained from mouth washings, showed nuclear polymorphism and enlargement of nuclear diameter. These changes in the cases with pernicious anemia disappeared rapidly by parenteral administration of vitamin B_{12}. Boen[2] accepted, therefore, these changes of oral squamous epithelial cells as characteristic

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of vitamin B_{12} deficiency.

In 1956 Gardner *et al.*[3] reported that the cases with tropical sprue showed abnormalities of squamous epithelial cells and gastric columnar cells as well as of their nuclei, which appeared to be similar to the changes associated with pernicious anemia, and these nuclear and cellular changes did not disappear rapidly with folic acid or vitamin B_{12} therapy as did the megaloblastic changes of the bone marrow.

Macrocytic anemia[4], which responded sharply to folic acid therapy but only slowly to vitamin B_{12} alone, has been found in not a few cases of children with nutritional dystrophy[5].

In the present investigation we attempted to examine the changes in diameters of nuclei of squamous epithelial cells obtained from mouth washings and their relation to deficiency of vitamin B_{12} on one side and that of folic acid on the other in children with nutritional dystrophy.

EXPERIMENTAL

MATERIAL AND METHODS

Estimation of vitamin B_{12} in blood :– Quantitative estimation of blood vitamin B_{12} was carried out according to the procedures which had been adopted by the Vitamin B Committee of Japanese Society of Vitaminology[6]. The method was outlined as follows. To 2.0 cc of heparinized whole blood, were added 2.0 cc of 0.2 M acetic acid buffer of pH 4.5, 0.1 cc of KCN solution (500γ/cc) and 15.0 cc of distilled water. The mixture was kept in a water bath at 100°C for 30 minutes. After cooling the content, 0.2 cc of 10% metaphosphoric acid solution was added and total amount of the content was brought up to 25.0 cc with an addition of distilled water. By centrifugation clear upper layer was obtained, and 10.0 cc of which was adjusted to pH 7.0 with N/50 NaOH solution, then the final volume was made up to 20.0 cc with an addition of distilled water. The clear fluid thus obtained was subjected to microbiological assay with use of Lactobacillus Leichmannii ATCC 4797.

Colorimetric estimation of formiminoglutamic acid in urine :— Formiminoglutamic acid in urine was estimated according to the description of Tabor *et al.*[7], which was a modification of the method used by Rabinowitz *et al.*[8] for formiminoglycerine determination. The estimation was done on 0.3 cc of the 24-hour urine.

Microscopic examination of nuclei of squamous epithelial cells from oral cavity :– The principle of the examination was similar to that used by Boen[2]. Children were asked to rinse their mouths three times with physiologic saline in order to remove food rests. Then approximate 5 cc of the saliva were collected into a centrifugal tube. Smears were prepared from the sediment obtained by centrifugation and were air-dried, then stained with the Giemsa

stain. The nucleus of squamous epithelial cells was measured with an ocular micrometer in both the largest and smallest diameters, and the average of these two figures was represented as "nuclear diameter" of the nucleus of each of the squamous epithelial cells. Nuclei of one hundred successive squamous epithelial cells were thus measured.

The children subjected to this investigation were 36 boys at the age of from 9 to 12 years and all of them showed angular stomatitis, cheilosis and glossitis as part manifestations of nutritional dystrophy[4]. They were divided into two groups. Group A (Cases 1–19) was given vitamin B_{12} in an amount of 25γ per day orally for successive eight months just prior to this investigation. Group B (Cases 20–36) was the control.

RESULTS AND DISCUSSION

The averages of "nuclear diameter" of the nuclei of squamous epithelial cells were 11.3±0.3μ and 11.7±0.5μ in Groups A and B respectively, showing no significant difference between these two figures (Cf. Tables I and II). This result was seemingly absurd, but it was not necessarily so, if the fact was taken into consideration that the majority of the cases of Group A remained in low levels of blood vitamin B_{12} (less than 0.3 mγ/cc) in spite of oral administration of vitamin B_{12} for a considerably long period. Then it was thought that it would

TABLE I. Diameters of Nuclei of Oral Epithelial Cells, Vitamin B_{12} in Blood, and Formiminoglutamic Acid in Urine of Children with Nutritional Dystrophy.

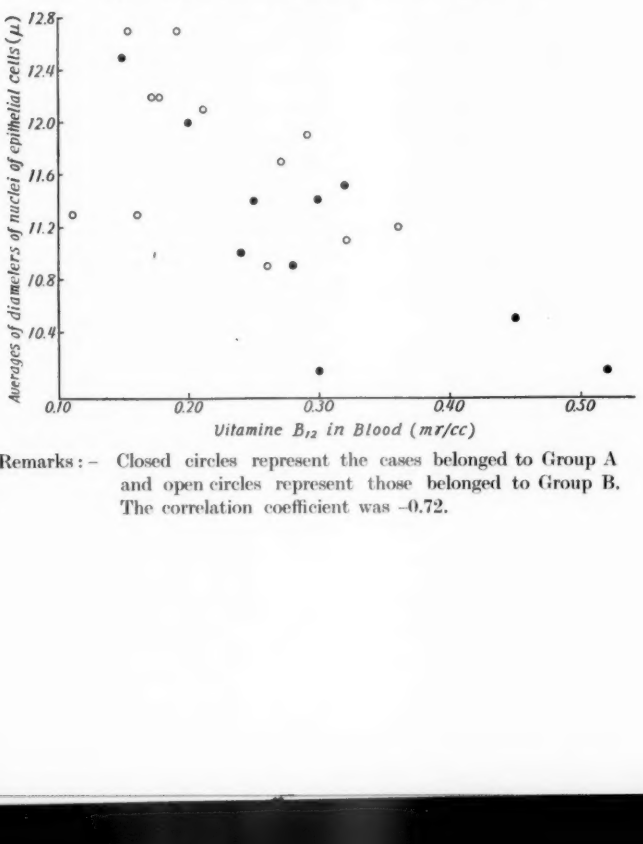
Group A.

No. of cases	Age (years)	Vitamin B_{12} in blood (mγ/cc)	Formiminoglutamic acid in urine (mM/0.3 cc)	Distribution of one hundred nuclei of oral epithelial cells according to their diameters (μ) 5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	Average of diameter of the nuclei in each case (μ)
1	9	0.30	0	0	0	4	5	15	14	19	13	11	10	4	1	2	0	2	11.4
2	9	0.30	0.115	0	0	3	17	24	15	13	10	10	5	2	1	0	0	0	10.3
3	9	0.38	0.068	0	0	1	3	8	8	15	17	25	11	6	4	2	0	0	12.2
4	9	0.45	0.037	0	0	2	9	13	15	21	17	15	5	2	1	0	0	0	10.5
5	9	0.52	0	1	1	4	16	23	14	16	7	7	6	4	1	0	0	0	10.2
6	10	0.18	0.048	0	0	1	3	4	8	20	16	17	11	10	4	3	2	1	12.5
7	10	0.18	0.045	0	0	0	2	12	11	17	18	17	13	7	3	0	0	0	11.9
8	10	0.33	0.068	0	0	2	9	13	15	20	12	12	9	6	2	0	0	0	11.2
9	10	0.23	0.031	0	0	1	6	7	7	18	21	19	10	8	2	0	1	0	10.9
10	10	0.20	0.030	0	0	2	7	14	17	19	10	13	10	6	2	0	0	0	11.3
11	11	0.24	0	0	0	2	6	15	17	22	19	9	8	2	0	0	0	0	11.0
12	11	0.30	0	0	0	3	12	26	21	16	11	7	3	1	0	0	0	0	10.1
13	11	0.15	0	0	0	1	2	6	8	14	17	17	17	10	6	1	1	0	12.5
14	11	0.19	0.042	0	0	3	5	12	13	20	18	16	8	4	1	0	0	0	11.5
15	12	0.32	0	0	0	3	6	13	14	17	18	17	7	4	1	0	0	0	11.5
16	12	0.45	0	0	0	3	8	13	17	16	17	18	6	1	1	0	0	0	10.5
17	12	0.28	0	0	1	3	7	18	17	17	14	14	5	4	0	0	0	0	10.9
18	12	0.20	0	0	0	2	4	11	11	20	14	14	6	7	5	6	0	0	12.0
19	12	0.25	0	0	0	1	9	18	15	13	15	10	9	4	4	2	0	0	11.4
Average		0.25±0.01																	11.3±0.3

TABLE II. Diameters of Nuclei of Oral Epithelial Cells, Vitamin B_{12} in Blood, and Formiminoglutamic Acid in Urine of Children with Nutritional Dystrophy.
Group B.

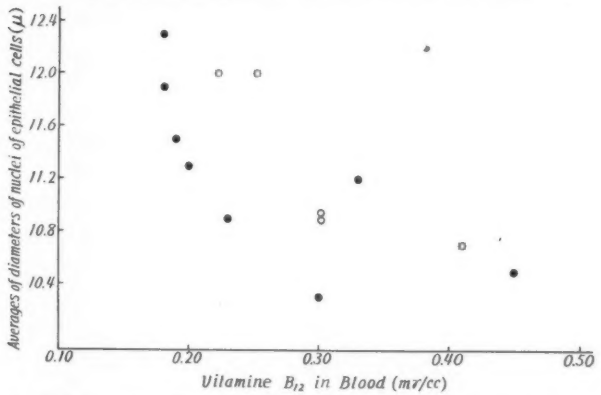
No. of cases	Age (years)	Vistamin B_{12} in blood (mγ/cc)	Formimino-glutamic acid in urine (mM/0.3 cc)	Distribution of one hundred nuclei of oral epithelial cells according to their diameter (μ) 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19	Average of diameter of the nuclei in each case (μ)
20	9	0.30	0.145	0 0 3 5 19 14 16 15 13 8 4 2 1 0 0	10.9
21	9	0.21	0.015	0 0 0 4 7 7 11 15 15 14 14 5 4 0 1	12.1
22	9	0.36	0	0 0 2 4 23 13 16 16 12 7 3 2 2 0 0	11.2
23	9	0.25	0.221	0 0 1 0 12 13 11 16 13 12 6 2 3 1 0	12.0
24	9	0.16		0 0 2 3 21 12 18 17 12 7 6 2 0 0 0	11.3
25	10	0.41	0.058	0 0 2 6 17 21 22 16 9 5 2 0 0 0 0	10.7
26	10	0.19	0.007	0 0 0 3 10 9 12 14 16 15 15 4 1 1 1	12.7
27	10	0.22	0.018	0 0 1 6 11 10 16 16 14 8 8 8 2 0 0	12.0
28	10	0.26	0	0 0 2 10 17 17 14 14 17 8 1 0 0 0 0	10.9
29	10	0.27	0	0 0 0 5 8 12 20 19 18 13 5 0 0 0 0	11.7
30	11	0.30	0.053	0 0 4 11 18 14 15 12 11 9 4 2 0 0 0	10.9
31	11	0.32	0	0 0 3 8 23 16 9 8 14 9 7 3 0 0 0	11.1
32	12	0.11	0	0 0 1 3 7 9 15 16 22 15 6 4 2 0 0	12.3
33	12	0.29	0	0 0 6 8 10 14 12 12 10 12 8 8 0 0 0	11.9
34	12	0.17	0	0 0 0 1 11 17 18 19 20 5 5 2 2 0 0	12.2
35	12	0.15	0	0 0 1 2 2 5 13 17 18 24 10 7 0 1 0	12.7
36	12	0.17	0	0 0 0 2 11 21 20 22 10 7 3 2 2 0 0	12.2
Average		0.23±0.02			11.7±0.5

Fig. 1. Correlation between Blood Vitamin B_{12} and Averages of "Nuclear Diameters" of Oral Epithelial Cells in Children with Nutritional Dystrophy without Marked Folic Acid Deficiency.



Remarks: – Closed circles represent the cases belonged to Group A and open circles represent those belonged to Group B. The correlation coefficient was –0.72.

Fig. 2. Correlation between Blood Vitamin B_{12} and Averages of "Nuclear Diameters" of Oral Epithelial Cells in Children with Nutritional Dystrophy with superposition of Marked Folic Acid Deficiency.



Remarks: – See Fig. 1. The correlation coefficient was –0.46.

be better to try to examine the correlation between the "nuclear diameter" of squamous epithelial cells and blood vitamin B_{12} level itself in one and the same individual, *irrespective of the vitamin B_{12} administration*. Of course mouth washing and blood taking should be performed at the same time. One more condition : Children with the urine positive to formiminoglutamic acid and those with the urine negative to it should be observed from each other, because the former were cases of folic acid deficiency, which might exert an influence upon the "nuclear diameter" of epithelial cells.[3] Fig. 1 shows the correlation between blood vitamin B_{12} levels and averages of the "nuclear diameters" of squamous epithelial cells in the cases negative to urinary formiminoglutamic acid (Cf. Fig. 1). In these cases the correlation coefficient was –0.72. Fig. 2 shows the same correlation in the cases positive to formiminoglutamic acid in urine. In these cases the correlation coefficient was –0.46 (Cf. Fig. 2). As is above shown, a significant reverse correlation – between vitamin B_{12} levels in blood and the "nuclear diameters" of squamous epithelial cells–was found only in cases– only males were used for the sake of convenience of urine collection– with nutritional dystrophy upon which folic acid deficiency was not superimposed.

According to Boen[2], nuclei with the diameter of 14.5 μ or more amounted to 26% of nuclei of successive 100 oral epithelial squamous cells in 7 cases of pernicious anemia, while the figures were only 5% or less in the control 21 persons. In our present investigation, the cases in which nuclei with the diameter of 15 μ or more amounted to 10% or more of successive 100 nuclei examined, were 13 in number (48.1%) out of 27 cases with 0.29 mγ/cc or less of blood vitamin B_{12},

while such cases were only 2 in number (28.5%) out of 7 cases with more than 0.3 mγ/cc of blood vitamin B_{12}.

SUMMARY

It was reported recently that diameters of oral squamous epithelial cells were influenced by deficiency of vitamin B_{12} or of folic acid. The existence of deficiency in vitamin B_{12} or/and folic acid was highly probable among the children with nutritional dystrophy in Hirosaki area. In 36 cases with nutritional dystrophy, the nuclear diameters of squamous epithelial cells were measured microscopically by using mouth washings, and their correlation to blood vitamin B_{12} levels, estimated microbiologically, was examined. At the same time superposition of folic acid deficiency was checked by testing formiminoglutamic acid in urine.

The correlation coefficients between blood vitamin B_{12} and the nuclear diameter of squamous epithelial cells were –0.72 in the cases without folic acid deficiency and –0.42 in those with it. Even though these two figures showed a not very significant difference, it may be said that the nuclear diameter of oral epithelial cells from nutritional dystrophy more closely correlated to blood vitamin B_{12} levels in the cases without folic acid deficiency than in those with it. In other words, folic acid deficiency, as was reported, also gave an influence on the nuclear diameter of epithelial cells, so a superposition of folic acid deficiency lessened the correlation coefficient between blood vitamin B_{12} levels and the nuclear diameters in our results.

CONCLUSIONS

The diameters of nuclei of oral squamous epithelial cells showed a significant reverse correlation ($r=-0.72$) to blood vitamin B_{12} levels in children with nutritional dystrophy upon which folic acid deficiency was not superimposed.

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Primary Varicella Pneumonia

By

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Varicella is usually a mild and benign disease and its severe complications are rare. Bullowa and Wishik[1] described 133 cases with complications among 2534 cases of the disease. These complications included secondary infections of vesicles, otitis media and pneumonia. According to Mitchell and Fletcher[2], the most frequent complications in 775 cases of varicella were furunculosis and otitis media, bronchopneumonia being the next in frequency. The above mentioned pneumonia associated with varicella were caused by secondary bacterial infections. Reports of primary varicella pneumonia are few in number and these reported cases were almost all adult cases.

In the present paper I shall describe the first report of primary varicella pneumonia in Japan; besides, this is about cases of children.

REPORT OF CASES

Case 1. Boy, aged 5 years and 10 months. He noticed a rash in his body and simultaneously complained of cough on July 8, 1957. The rash spread rapidly over his whole body and coughs increased in frequency and in intensity. He visited our Hospital on July 10, 1957. On examination, he was slightly malnourished and his skin showed characteristic varicella macules, papules and vesicles over the face, the chest, the abdomen, the extremities, the palmes and the soles. These papules and vesicles were partly confluent. The pharynx was reddened and vesicles were found in the mouth. Heart sounds were clear. Examination of the chest revealed no dullness over the lungs on percussion, where only somewhat roughened breath sounds were heard. The liver and the spleen were not palpable.

Course. Vesicles and papules increased in number and on July 11 fever rose up to 39.5°C and coughs were still intense. Physical examination of the chest did not show any striking change and he was not dyspneic as not on the previous day. On July 12, when he came to our Hospital, fever was 37.1°C,

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but rose up to 39.5°C on this evening. On July 13, when he came to us, fever was 36.7°C and coughs became less frequent. On July 15, no fever was noticed and he coughed hardly ever. Tetracycline was given for three consecutive days from July 12 on. Roentgenogram of the chest showed in the lower part of the right lung a shadow adjoining the heart and the diaphragma on July 10 (Fig. 1). This shadow was not seen any more on July 18 (Fig. 2).

Laboratory findings. As for blood picture, on July 13 red cell count was 4,700,000 per cmm, hemoglobin 83 % after Sahli's method, and white cell count was 19,800 per cmm. Neutrophils were 20.0% lymphocytes 75.0 % and monocytes were 5.0 %. Platelet count was 360,900 per cmm. Tuberkulin test was negative. Red sedimentation rate was 24 mm on July 11, and 11 mm on July 18 in the first hour. The titer of cold agglutinin was on July 11, 1 : 32 on

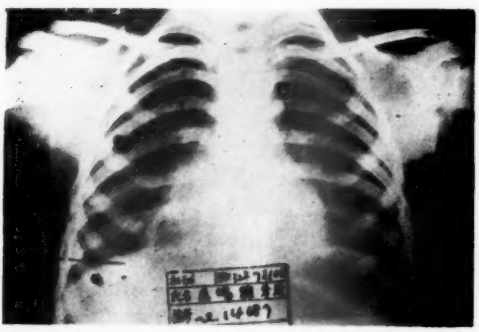

Fig. 1. Roentgenogram of the chest of Case 1. Shadow in the lower part of the right lung adjoining heart and diaphragma.

Fig. 2. Roentgenogram of the chest of Case 1, 8 days later. No more shadow.

July 18, 1 : 2. Urine was normal.

Case 2. Boy, aged 3 years and 3 months. A rash with fever was noticed on June 7, 1956. The rash spread rapidly over the whole body and he complained simultaneously of cough. He visited our Hospital on June 8, 1956. On examination, he was moderately nourished and his whole body was covered with numerous characteristic varicella macules, papules and vesicles. Heart sounds were clear. On the chest and the abdomen no abnormalities were found except the rash. His temperature was 37.5°C.

Course. On June 9, he was still feverish. On June 10, he complained of high temperature in the morning and he seemed tired. Coughs became more and more intense. Crepitant rales were heard over the whole lungs and on the lower part of the left lung fine moist rales were heard. On June 13, fever abated and the rales over the chest became almost inaudible. The coughs also diminished. Roentgenogram of the lungs showed a swelling of both hili and spotted shadows in the right lung, specially in the lower part of it.

Laboratory findings. As for blood picture, on June 10, red cell count was 3,850,000, hemoglobin 85 % after Sahli's method, and white cell count was 8,800. Neutrophils were 55.0 %, lymphocytes 30.5 %, monocytes 10.5 %, and virocytes were 4.0 %, Red sedimentation rate was 32 mm in the first hour.

Case 3. Boy, aged 1 year and 6 months. Fever was noticed with varicella rash and cough on December 15, 1956. Vesicles, papules and macules increased in number and the coughs became more and more intense. He visited our Hospital on December 17, 1956. On examination, he was moderately nourished and his whole body was covered with numerous characteristic varicella macules, papules and vesicles, and these papules and vesicles were partly confluent. Slight dyspnea and movement of alae nasi were noticed. Heart sounds were clear. Pulse rate was increased. The roughened breath sounds were heard over the lungs. The pharynx was reddened.

Course. On December 18, his general condition improved, and then fever abated. Coughs diminished day by day. Red sedimentation rate on December 17 was 18 mm in the first hour. Roentgenogram of the lungs showed increased density of both hili, spotted shadows in the middle of both lungs and a diffuse infiltration near the left hilus.

COMMENT

The clinical features of the present three cases will be summarized as follows :

1) Coughs occurred almost simultaneously with varicella rash and increased gradually in frequency and in intensity.

2) The cough was very intense and obstinate.

3) The rash was extensive, and eruptions were numerous. Eruptions were found also in the mouth. Fever was high and the general condition was

considerably bad.

4) The physical findings of the chest were slight, though the roentgenogram showed remarkable findings.

5) In the blood picture, though leucocytosis was noticed in Case 1, there was also lymphocytosis. In Case 2, leucocytosis was not noticed.

6) Cold agglutination test was negative in Case 1.

The above mentioned features will indicate that the pneumonia of the present three cases was not caused by any secondary bacterial infection, but by varicella virus itself.

Reports of primary varicella pneumonia in childhood are few in number. Lucchesi *et al*[3]. and Oppenheimer[4] reported on the basis of autopsy findings pneumonic affection in cases of varicella neonatorum. Except these reports about the newborn, there was only a case of varicella pneumonia in childhood — the case reported by Waldman[5]. On the basis of literature survey and my own experience, varicella pneumonia seems to be milder in the childhood with the exception of the newborn than in the adult.

SUMMARY

Three cases of primary varicella pneumonia were reported. Clinical features and laboratory findings of these three cases revealed that pneumonias of these three cases were all caused by varicella virus itself. This is the very first report of primary varicella pneumonia in Japan.

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Hypergammaglobulinemia and Its Relation to Ascites in Tuberculous Peritonitis

By

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Analysis of serum protein by means of electrophoresis made a great contribution to the medical progress. It is well known that hypergammaglobulinemia occurs in neoplasms and infections, specially in myeloma and macroglobulinemia.

In the present paper I shall report hypergammaglobulinemia in a patient of tuberculous peritonitis whose ascites also revealed a high level of gammaglobulin.

REPORT OF CASE

A girl, aged 11 years and 7 months, complained of fatigue, anorexia and fever on May 1, 1957, in the afternoon and occasional bloody sputa had been seen for about ten days, and a distension of the abdomen for five days. She had had occasionally abdominal pain since the summer of two years ago. She evacuated once a day, and stools were mostly of normal consistense, but occasionally loose or hard. She was admitted to our Hospital on May 11, 1957, under the provisional diagnosis of tuberculous peritonitis. On examination, she was malnourished and her temperature was 37.1°C. Heart sounds were clear. No abnormality was found physically over the lungs. The abdomen was considerably distented and fluctuation was noticed. The liver and the spleen were not palpable. The pharynx was slightly reddened. Slight edema was noticed on the legs. Her weight was 37.6 kg.

Course. Fever rose up to 37.8°C and remained to persist for two days after admission, and then she had occasional attacks of fever up to 37.5°C. Streptomycin, para-aminosalcylic acid and isonicotinacid hydrazid were administered from May 18, 1957, on. Removal of ascitic fluid was performed twice a week in the early time of admission and about 20–200 cc of ascitic fluid was obtained every time. After removal of ascitic fluid, 20 mg. of isonicotinacid hydrazid was infused into the peritoneum. By the above mentioned treatment,

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ascitic fluid gradually decreased and abdominal pain was diminished. By the end of June, distension of the abdomen was no more noticed, and then removal of ascitic fluid and infusion of isonicotinacid hydrazid were suspended. The general condition became much better and the body weight increased. Appetite became also better. She was discharged on September 6, 1957. It will be mentioned here that no pathological changes were found in roentgenograms of the chest and the abdomen at the admission.

TABLE I. Blood Picture of My Own Case of Hypergammaglobulinemia.

Blood picture \ Date		May 11	June 20	August 5	September 5
Red cell count		3,570,000	4,040,000	4,340,000	4,890,000
Hemoglobin level (by Sahli's method)		87%	110%	77%	—
White cell count		6,000	4,400	3,200	5,000
Percentage (%)	Eosinophils	1.0	0	0.5	1.5
	Neutrophils	66.5	44.5	35.5	40.5
	Lymphocytes	27.0	51.5	63.0	58.0
	Monocytes	5.0	0	1.0	5.0

TABLE II. Red Sedimentation Rate of My Own Case of Hypergammaglobulinemia.

Date	The first hour	The second hour
May 11	82 mm	109 mm
June 10	50 mm	98 mm
July 15	13 mm	47 mm
August 10	15 mm	38 mm
September 2	8 mm	16 mm

TABLE III. Paper Electrophoretic Analysis of Serum Protein and Ascitic Fluid of My Own Case of Hypergammaglobulinemia.

		Total protein	Albumin	Globulin				A/G
				α_1	α_2	β	γ	
Serum	May 11,1957	8.57 g/dl	24.5%	8.5%	10.9%	7.6%	48.2%	0.32
	May 28, "	9.77 g/dl	29.5%	6.2%	9.5%	10.6%	44.2%	0.42
	June 6, "	9.52 g/dl	33.7%	5.1%	9.0%	9.4%	42.8%	0.51
	June 17, "	9.01 g/dl	35.1%	6.4%	8.8%	10.2%	39.5%	0.54
	April 1,1958	7.5 g/dl	42.7%	6.5%	9.1%	13.6%	28.1%	0.75
Ascites	May 28,1957	7.07 g/dl	32.3%	5.3%	7.8%	7.4%	47.2%	0.47
	June 6, "	7.42 g/dl	33.9%	6.6%	6.2%	9.2%	44.1%	0.51
	June 12, "	7.68 g/dl	46.8%	4.9%	4.4%	8.5%	35.4%	0.88

Laboratory findings. As for blood picture, as is given in Table I, slight anemia was noticed on her first visit to our Hospital. Red sedimentation rate was examined each week during her admission. As is given in Table II, the sedimentation rates of about normal value were seen on July 15th and thereafter. Wassermann reaction of serum was negative. Tuberculin test was positive. Blood pressure was 110/80. In smears and cultures of sputum and ascites, no tuberculous bacillus was found. Liver function test on May 23, revealed 5‰ of Weltmann's coagulation band and two weeks after, 4 % of bromsulfalein test and 4 of icterus index. Takata's test and cryoglobulin were negative. Analysis of serum and ascites by means of paper electrophoresis, as is given in Table III, revealed high levels of gammaglobulin in both serum and ascites. Ascites was yellowish, clear and serofibrineous. In urine and feces no pathological findings were noticed.

DISCUSSION

Feinstein and Petersdorf[1] examined 394 cases of hyperglobulinemia, of which 268 were cases of multiple myeloma, sarcoidosis, collagen disease, liver disease, carcinoma and chronic pulmonary diseases. And 20 % of these patients with hyperglobulinemia had hyperproteinemia. My own case had hyperglobulinemia with hyperproteinemia too. Leonhardt[2] suggested that hypergammaglobulinemia might precede the other manifestations of systemic lupus erythematosus. As to patients with marked hypergammaglobulinemia without signs of myeloma or macroglobulinemia, Waldenström[3] suggested a condition which might be designated as premyeloma. He stated that a high sedimentation rate should be present for some years before other signs of myeloma appear, if there was a premyelomatous state. But sometimes it may, as he further states, be benign hyperglobulinemia of obscure origin to which he gave the name "essential hyperglobulinemia".

Now my own case had, as has been described, hypergammaglobulinemia too, and though it is with a good reason to ascribe the hypergammaglobulinemia to the tuberculous peritonitis she had suffered from increased levels of gammaglobulin of serum and ascites in my own case seem to be abnormally high. The electrophoretic patterns of serum protein and ascitic fluid were similiar. It is difficult to absolutely exclude the possibility that my own case may develop to multiple myeloma or macroglobulinemia, but the very fact that she has been well for these two years since discharge will indicate that the hypergammaglobulinemia of my own case might have been due to chronic infection of tuberculosis.

SUMMARY

A case of hypergammaglobulinemia associated with tuberculous peritonitis was presented. The electrophoretic patterns of serum protein and ascitic

fluid of the patient were similar and presented abnormal high levels of gammaglobulin. It was thought more natural to attribute the hypergammaglobulinemia to tuberculous infection than to premyeloma or such conditions.

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Localized Response of the Toad's Retina to Microillumination and Intermittent Light

By

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INTRODUCTION

Recent development of microelectrode technique has made possible a recording of a potential localized within a small part of tissue. Applying this technique to the retina, Tomita[1)2)] recorded potentials from various depths of the retina and called them "intra-retinal electroretinogram" (EIRG). Svaetichin[3)], Brindley[4)5)] and other workers[6)7)8)] also have tried to analyse retinal response by similar methods. But it has remained to be explored how these potentials of different layers are related with one another.

In the present experiment potential changes were recorded at the same time from two different layers, that is, the surface of the receptor layer and the inner plexiform layer, and compared with each other.

METHOD

Experiments were made on retinae of toads (Bufo vulgaris). Preparations were made under weak daylight. After removing the pigment epithelium the retina was mounted with the receptor side upwards on a piece of Ringer-soaked black cloth covering Ag-AgCl plate which served as an indifferent electrode. Two similar KCl-filled micropipettes about 1 μ in tip diameter were used as recording electrodes. The potential changes were led to the grids of a two channel preamplifier and displayed on a dual beam cathode-ray oscilloscope through a C-R coupled main amplifier. Each element of amplifier was adjusted to be similar in gain and to the same time constant, 0.9 sec. A light spot about 250 μ square and 1200 lux in intensity was used as a stimulus, its duration being about 0.7 seconds. In a part of experiments this small light spot was interrupted by means of a rotating sector of light-dark ratio 1 : 1. All the experiments were carried out at room temperatures of 20–25°C.

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RESULTS

Limited extent of potentials to microillumination

When two microelectrodes were placed at two neighboring points of the receptor surface, the potentials recorded by these electrodes in response to the light stimulus were almost identical (Fig. 1 A). The positivity of the electrode was directed upwards. When one of the electrodes was advanced into the retina to a depth of 180–200 μ from the receptor surface, a large sharp negative potential appeared on illumination and was occasionally followed by a smaller and slower negative potential (Fig. 1 B).

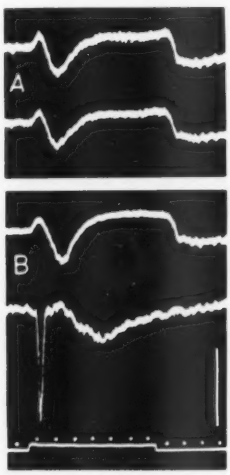

Fig. 1. Simultaneous recordings of retinal responses to microillumination.
A: Identical responses obtained by two similar microelectrodes placed at two neighboring points on receptor surface of an inverted retina.
B: Upper beam indicates response similar to A, but lower beam sharp negative response obtained with one electrode advanced into inner plexiform layer. Positivity upwards. Vertical bar indicates 500 μV and time mark 10 cps.

The response picked up from the receptor surface will be called the "response R" to be distinguished from the response picked up at the deeper layer or the "response D". In all the figures of this paper the upper beam of each record represents the response R and the lower one the response D. The preceding sharp on-potential of the response D was in some cases smaller and more prolonged in duration, and this seemed to be a sign of deterioration, because it was observed more frequently in aged preparations. Müller-Limmroth and Güth[9] obtained similar potentials from the frog's retina.

The record shown in Fig. 2 A was obtained when the retina was illuminated in

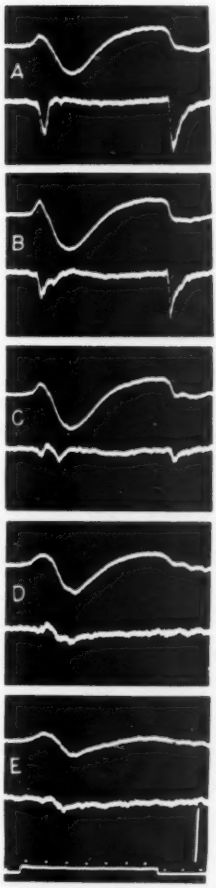

Fig. 2. Difference of responses according to relative position of the stimulus light spot to the recording electrodes. Light spot was centered on electrodes for A and was moved step by step for B—E. Vertical bar 500 μV, time mark 10 cps.

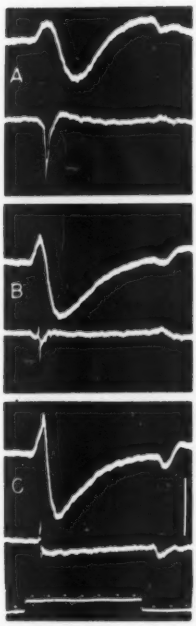

Fig. 3. Size effect of stimulus light spot on both responses. Size was 250 μ square for A, 1200 μ square for B and even illumination for C, otherwise as in Figs. 1 and 2.

such a manner that the two microelectrodes about 20 μ apart were at the center of the light spot. Then a spot of light was moved step by step along a horizontal line passing midway between the two electrodes, and for each position of the light spot responses were recorded. The responses shown in B were recorded when the edge of the square light spot was going to leave the electrodes. The response R was similar to that in record A, but the sharp on-potential of the response D

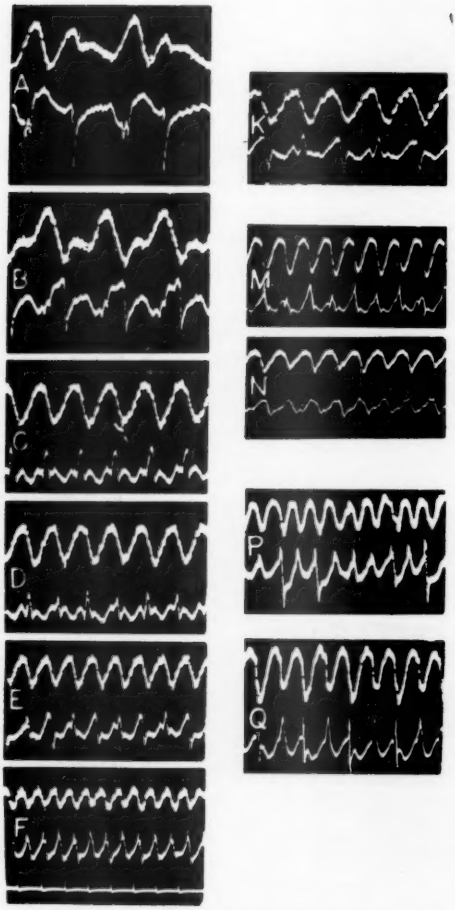

Fig. 4. Upper and lower records are responses to intermittent light. Explanation in text. Time mark 10 cps.

was reduced in amplitude. The light spot was moved farther away with 100 μ steps and records C and D were obtained. The record E was obtained at a distance 200 μ farther away. It is apparent from these records that the sharp response D is a sharply localized phenomenon, while the extent of the response R is more diffuse. Brindley[5] also found sharply localized responses in deeper layers of the retina.

Next, the effect of the stimulus size was investigated on both sorts of

responses. When the size of the stimulus was increased above a certain limit, — say 800 μ square — the sharp response D showed a marked decrease in amplitude, while the response R became more prominent and sharper (cf. A with B in Fig. 3). The record C in Fig. 3 was obtained by even illumination. The response D is quite different in shape from the control record A : It consists of a small positive-negative spike and a small steady negative deflection.

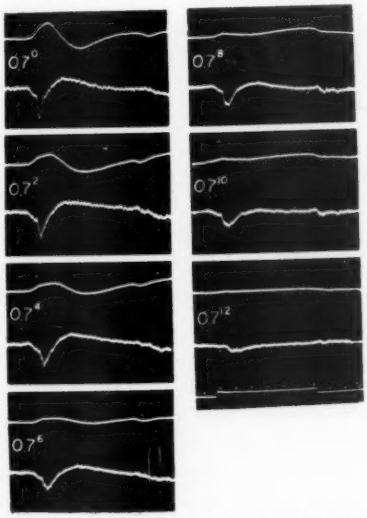

Fig. 5. Responses to various intensities of stimulus light. Numerals indicate transmission factors of neutral tint filters used for reduction of intensity, 1200 lux for 0.7°.

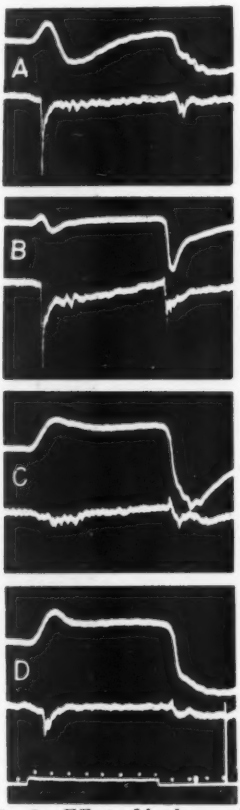

Fig. 6. Effect of background illumination on responses. Responses were obtained in dark adaptation at A, under background illumination of 30 lux at B, immediately after turning off of background illumination at C and farther recovery at D.

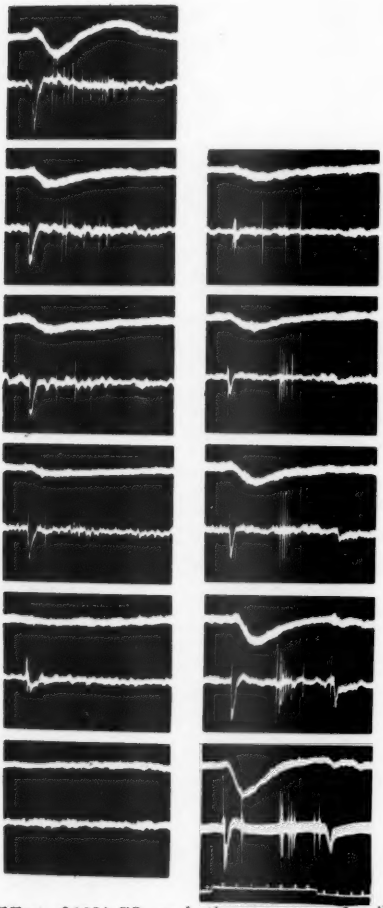

Fig. 7. Effect of 10% CO_2 on both responses and spike discharges. Left column shows responses recorded every 20 seconds after application of CO_2 and right column shows recovery process.

Responses to intermittent light

In Fig. 4 responses to intermittent light of various frequency are illustrated. In experiment with steady illumination the positive phase of the response R was in phase with the negative deflection of the response D. In intermittent stimulation a phase shift occurred so that the negative phase of the response R corresponded with the sharp negative response D (see B). When the rate of flicker was raised further the sharp response D became positive (see C, D, E and

F). It happened also sometimes that a negative and a positive sharp response Ds appeared alternately (see record K),or in an irregular order (see P and Q). In contrast to the response D, the response R was generally more regular, although alternation was found in some cases (see Q). In a certain preparation alternating waves appeared in dark adaptation, as shown in M, but they changed into regular waves with light adaptation, as shown in N.

Independent variations of both responses under various conditions

Fig. 5 illustrates the effect of reducing the intensity of stimulus light. The intensity is indicated by 0.7^n, transmission factor of the neutral filter. It is 1200 lux at 0.7°. As the intensity was lowered the response R was reduced more rapidly in amplitude than the response D. For example, at 0.7^8 the response R was reduced to about one eighth of the control at 0.7°, while the response D was half the control.

The effect of light adaptation is shown in Fig. 6. Record A is obtained under dark adaptation and record B under background illumination of 30 lux. At this level of light adaptation the on-deflection of response R was much smaller than in A, while the sharp on-potential of response D was as large as in A. Immediately after turning off the background illumination, the sharp negative deflection of response D disappeared, and recovered with time thereafter (see C and D).

Fig. 7 illustrates the effect of 10% CO_2 on both responses and spike discharges. The left successive records were obtained about every 20 seconds since 10 % CO_2 began to stream at a rate of 2 l/min. into the box in which the preparation was placed. The number of spikes and the amplitude of the response R were diminished progressively, but the response D remained about unchanged over a long period and survived the other responses at the last stage of CO_2 action. In the right column the recovery process is shown.

DISCUSSION

From the results shown above it is obvious that the response D is different from the response R in many respects. The retinal response to even illumination can not be represented as a sum total of responses to small illuminations. The response R was obtained from the receptor surface and contained no such sharp element as the first component of the response D which was obtained from a more proximal layer (probably the inner plexiform layer). One may be tempted to interpret the response R as a response closely related to the activity of the receptor cells, and the response D as a response concerning the activity of proximal network. It seems, however, to be difficult to explain from this point of view the following differences observed between the responses R and D: 1. The response D seemed to be more sensitive to light than the response R (see

Fig. 5). 2. Response D seemed to be more resistant to light adaptation than response R (Fig. 6). 3. Response D survived response R and spike discharges when the retina was subjected to the action of 10% CO_2 (Fig. 7).

Dodt and Enroth[10] recorded retinal responses to intermittent light with special reference to progressive variation of a, b, and d waves of the ERG, and discussed the roles of cones and rods in the ERG-generation. It may be supposed that the sharp response D reflects the activity of cones, while the slow response R concerns the rod activity, because the former is more resistant to light adaptation than the latter. If this interpretation is correct, it may be expected that the critical fussion frequency of flicker (CFF) is higher for the response D than for the response R. A series of experiments was carried out to determine the CFFs of both responses. In reality, however, no definite difference could be found between the CFFs of both responses.

SUMMARY

Slow potentials to microillumination ($250\times250\ \mu$) were recorded at the same time with KCl-filled microelectrodes from the surface of the receptor layer and the inner plexiform layer of an inverted retina preparation of a toad. The surface response (response R) consisted of a slow positive- negative deflection, while the deep one (response D) consisted of an initial sharp negative and a second slower negative deflection.

1. The response D was more localized than the response R.
2. Both responses showed phase shifts during intermittent stimulation. Critical fusion frequencies were almost identical for both sorts of responses.
3. They varied independently as the intensity of illumination and the adaptation level were altered.
4. 10 % CO_2 abolished first the response R and then the response D.

I am greatly indebted to Prof. K. Motokawa for his kind guidance and for many valuable discussions.

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Hypothermia and Cortical Evoked Potentials

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INTRODUCTION

The thalamo-cortical relationship has been studied by many workers[1–7] on the basis of recruiting responses since this phenomenon was discovered by Morison and Dempsey.[1]

The recruiting responses can be recorded widely on the cortical surface, especially of the frontal area, by repetitive stimulation of the centrum medianum, the intralaminar nuclei, the nucleus ventralis anterior, the anterior pole of the reticular nucleus and the nuclei of the medial portion of the thalamus.

On the other hand, induced hypothermia has been investigated with special reference to spontaneous electrical activity and evoked potentials of the cortex.

It is reasonable to suppose that the recruiting response depends on metabolic processes of cortical and thalamic cells and will be influenced greatly by the temperature of the brain.

The purpose of the present experiments is to investigate the effect of hypothermia on the recruiting response.

METHOD

Albino rats were used under light anesthesia with sodium pentobarbital injected intraperitoneally.

The brain surface was exposed widely, and a stimulating electrode was inserted stereotaxically[8] into the nucleus ventralis anterior or the anterior pole of the nucleus reticularis of the thalamus. The stimulating electrode was a bipolar concentric electrode consisting of stainless steel wire 250 μ in diameter, the tip of which was protruded about 1 mm. Through this electrode, repetitive stimuli, square pulses of 0.3 msec. in duration and 1.3–2.6 volts in intensity, were given for about 5 sec. The frequency of repetition ranged from 0.5 to 20 cps.

The response was bipolarly picked up chiefly from the frontal cortical surface and partly from the parietal, and recorded by an ink-writing electroence-

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phalogragh, where a cathode-ray oscilloscope served as a monitor.

The brain temperature was measured by means of a copper-constantan thermo-junction inserted into a depth of 2 mm. from the surface.

Next, the animal was immersed into ice-water except for the head, to record recruiting responses during the course of gradual cooling. When the brain temperature fell down to 18°C. the body was rewarmed gradually, replacing the ice-water by progressively warmer water. The rate of cooling was not exactly uniform, but was of the order 1 degree per five minutes. The rate of rewarming was made about the same as that of cooling. After each experiment, the thalamic nuclei stimulated were identified macroscopically as well as histologically.

RESULTS

a) Recruiting response

The stimulating electrode was inserted into the thalamus step by step, and

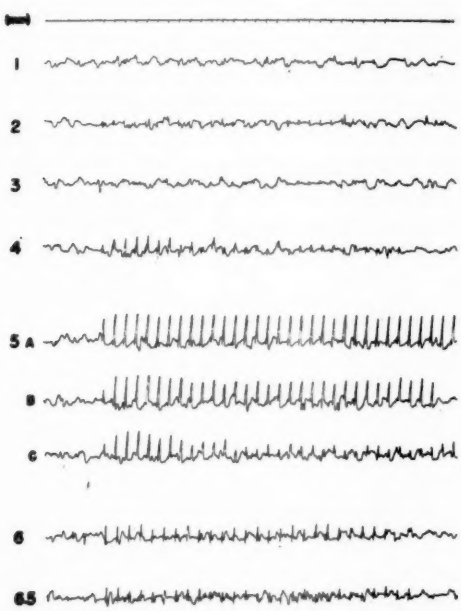

Fig. 1. Recruiting responses obtained when stimulating electrode was inserted into various depths from cortical surface. Numerals on left indicate depths in mm. 5–A, B and C were obtained by stimulation at 1.9, 1.3 and 0.9 volts respectively. Calibration for voltage and time same as in Fig. 3.

at each position test stimulation at 6 cps. and 1.9 volts was carried out.

At first, no response appeared on the ipsilateral frontal cortex, and a sign of recruiting response began to appear when the electrode tip reached a depth of approximately 4.5 mm. from the cortical surface. The most vigorous responses were obtained at a depth of 5 mm. from the cortical surface. The stimulating electrode was fixed at this depth, and the following experiments were performed.

When the stimulating voltage was too high, the waxing and waning property characteristic of the recruiting response was not clear; the response appeared in constant amplitude after initial waxing. But on reducing the stimulating strength the recruiting response appeared in its characteristic form — waxing and waning — as shown in Fig. 1-C. In this record, the amplitude of each response increased till the fourth response, and then decreased gradually.

In general, it is said that the maximal amplitude in recruiting response is attained in from the third to the fifth response.[1)]

If the stimulating electrode was advanced further, the responses were diminished in amplitude or could no longer be recorded (see two records at bottom in Fig. 1).

Observed on the cathode-ray screen, each response consisted of a small positive deflection and a large negative one which seemed to develop in two steps, the polarity referring to the electrode on the stimulated side.

b) Hypothermia and spontaneous electroencephalogram

The spontaneous EEG of the anesthetized rat in hypothermia was investigated by Ten Cate, Horsten and Koopman[8)] and Battista[10)]. In their experiments, the amplitude of EEG decreased as the temperature was lowered and disappeared at 18°C.–16°C.

In the present experiment, spindle bursts like the well-known barbiturate spindle made their appearance during cooling as well as during rewarming when the brain temperature remained within a range from 29°C. to 24°C. (Fig. 2).

Otherwise, the change of EEG was similar to those found by the previous investigators; the background activity was reduced in amplitude and slowed in rate with lowering temperature, and flattened at 19°C.–18°C.

c) Recruiting response and hypothermia

In the experiments shown in Figs. 3–6, the optimal frequency of stimuli was measured at each temperature. In this determination the optimal frequency was defined as the rate of stimulation at which the amplitude of the "typical" recruiting response was maximal. The type of response was called "typical" when the amplitude of response increased continuously, attained a maximum and then decreased steadily. At 37°C. a stimulus of 10–12 cps. was most

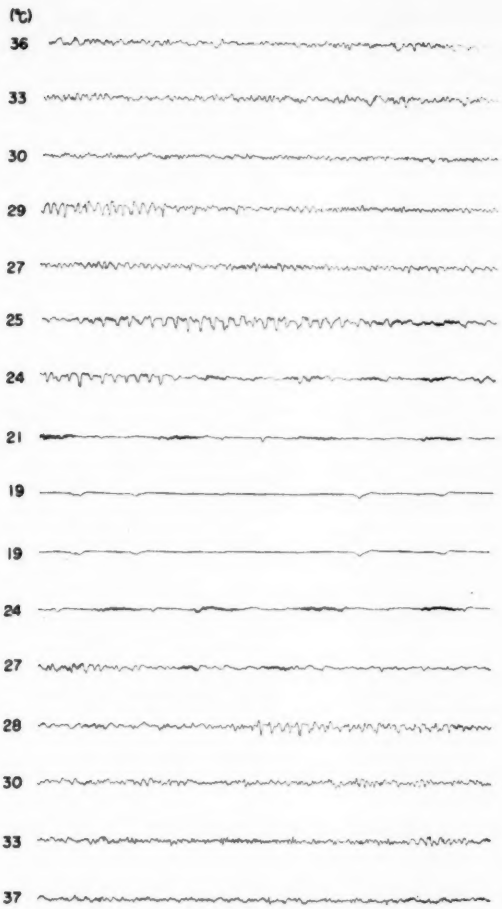

Fig. 2. Spontaneous EEG during temperature changes from 36°C. to 19°C. and in reverse direction. Calibration for voltage and time same as in Fig. 3.

adequate to elicit recruiting response. The optimal frequencies at about 30°C. and at 24°C. were 8–6 and 3 cps. respectively.

Thus, it is apparent that accompanying reduction of the brain temperature the optimal frequency becomes lower.

In general, the type of response was "typical" when the frequency of stimulation was much higher than the optimal frequency. But when the frequency was a little higher than the optimal one the amplitude of response

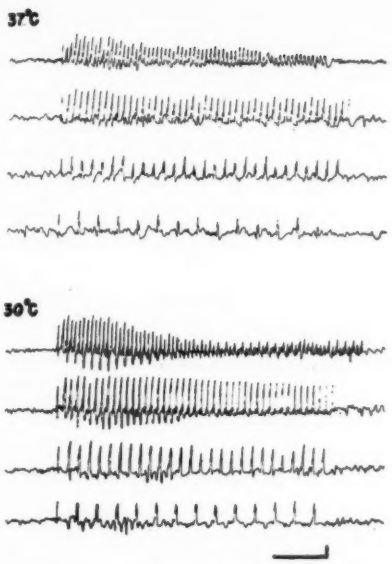

Fig. 3. Patterns of recruiting responses to stimulation at rate of 13, 8, 6 and 3 per sec. (from top downwards) at levels of temperature 37°C. and 30°C. in course of cooling. Calibration ; 1 sec. and 500 μV.

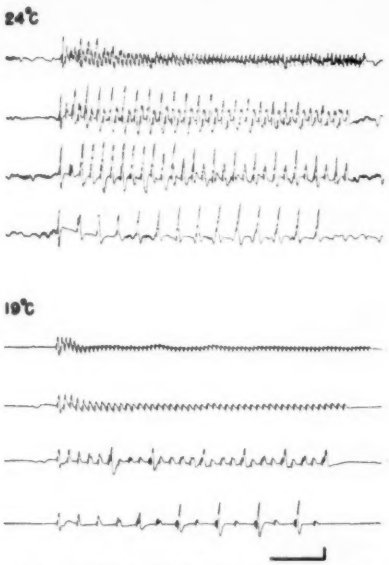

Fig. 4. Same as in Fig. 3 at temperature levels 24°C. and 19°C. in course of cooling.

changed alternately. For frequencies intermediate between the two cases mentioned above is also occurred every four responses (see the second record from bottom in Fig. 4).

Between the logarithm of these optimal frequencies and the brain temperature, there was linear relationship as shown in Fig. 7, although some deviation occurred in the temperature range near normal temperature. In this figure showing the results observed from three materials, three continuous curves show close agreement, indicating that there is little individual difference concerning the optimal frequency. The broken curves in the same figure refer to experiments of rewarming.

In one case (CASE 2) the velocity of rewarming was so rapid that the optimal frequency of stimulation tended to be higher than in the other cases, but the trend of the curve was the same as that of the others.

From this linear relationship between the logarithm of the optimal frequency and the brain temperature, the law of Arrhenius seems to be applicable to this phenomenon.

The temperature characteristic (μ) was calculated according to the formula

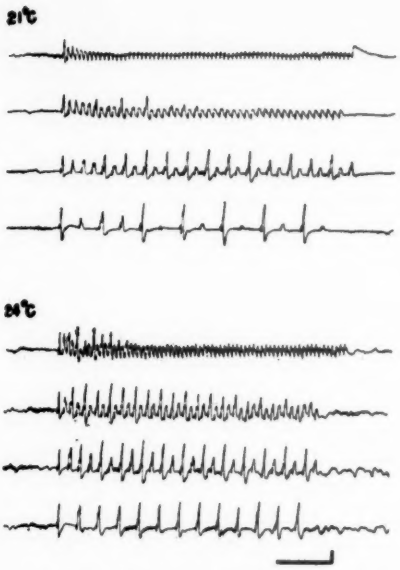

Fig. 5. Same as in Fig. 3 at 21°C. and 24°C. in course of rewarming. Alternation is conspicuous at 24°C.

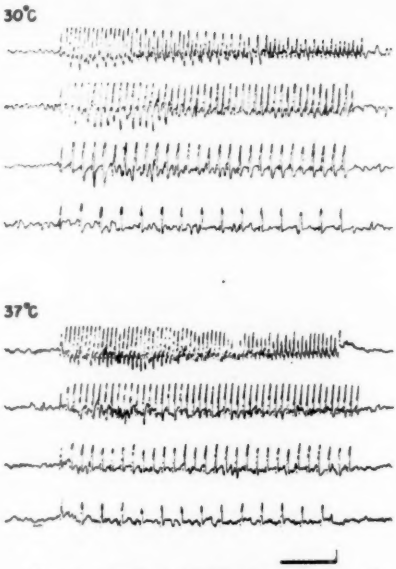

Fig. 6. Same as in Fig. 3 at 30°C. and 37°C. in course of rewarming.

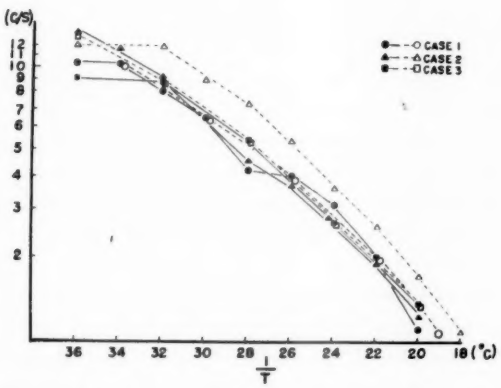

Fig. 7. Relation between logarithm of optimal frequencies and brain temperature. Continuous curves refer to cooling and broken ones to rewarming.

of Arrhenius, and a value of about 7500 cal. was obtained.

DISCUSSION

In 1942 Morison and Dempsey[1] found that rhythmic stimuli applied to the internal medullary lamina or dorsomedial thalamic region led to the appearance of recruited potentials in essentially all regions of the homolateral cortex, especially gyrus proreus, anterior sigmoid gyrus and middle and posterior suprasylvian gyri in cat.

Starzl and Magoun[6] reported that the nucleus ventralis anterior was the single most powerful recruiting nucleus, and that excellent recruitment was given by stimulation of the anterior pole of the reticular nucleus, though stimulation of the centre médian and intralaminar nuclei was also effective in eliciting recruiting responses in cat.

They described also the so-called augmenting response which is similar in appearance to the recruiting response, but different from it in various aspects. The so-called augmenting response was elicited from the nucleus ventralis lateralis, and could be observed principally in the somatosensory cortex.

In the present experiments the stimulated portion of the thalamus was the nucleus ventralis anterior or the anterior pole of the reticular nucleus, but not the nucleus ventralis lateralis. Moreover, the response was not restricted to the sensory-motor cortex, but distributed widely on the frontal, motor and somatosensory areas. Therefore it is likely that the responses obtained in these experiments were the recruiting response. It is, however, to be noted that in cat[6] and monkey[11] the recruiting response can appear in relatively restricted areas as compared with in rat.

In the present experiments the responses could be elicited by such a low voltage as 1–2 volts, and this fact also suggests that they were the recruiting response.

The cortical spontaneous activity of the rat during hypothermia disappeared at 18°C. of the brain temperature in agreement with the previous experiments. In Battista's experiments[10] the EEG flattened between 18°C. and 16°C. Ten Cate, Horsten and Koopman[9] reported also that the spontaneous activity of the rat disappeared virtually at 20°C.–18°C. of rectal temperature. The only difference between the present results and those of the previous workers consists in that the spindle burst appeared clearly between 29°C.–25°C., whereas no such burst was observed by the previous investigators. The appearance of spindle burst was observed also in dogs in a temperature range 29°C.–25°C. (unpublished). This range of temperature seems to coincide with the temperature range in which the recruiting response appeared with the maximal amplitude (see Fig. 8).

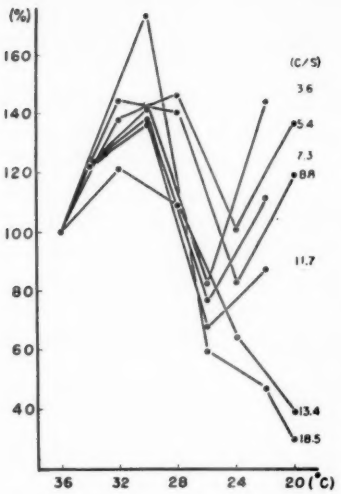

Fig. 8. Hyperresponsiveness at subnormal temperature. Ordinates; Amplitude of response in percentage of normal. Numerals on right indicate frequency of stimulation.

This relation is important in two senses; it suggests firstly that there is something common to electrogenesis of both recruiting response and spindle burst, and it indicates secondly that the brain shows a phase of hyperresponsiveness in some range of subnormal temperature.

The amplitude of the dorsal root potential of cat was maximal in a certain range of subnormal temperature in the experiments by Suda, Koizumi and Brooks,[12] and a similar phenomenon was observed by Battista[10] concerning the evoked potential of rat's cortex in response to sciatic nerve stimulation. Suda et al. interpreted this phenomenon as a result of synchronization of fiber action potentials.

It has been shown above that alternation occurs in some range of frequency above the optimal one (see, Fig. 5).

The negative deflection of each response seems to consist of two components, the one of which develops earlier and more slowly, and the other develops later and more rapidly. The latter is more liable to the effect of lowering temperature probably because its faster development requires a higher rate of metabolism. It is the late component that drops out in the phenomenon of alternation.

The mechanism of alternation is not clear, but it may be supposed that transmission from the early component to the late one is blocked somewhere in the recruiting system when the frequency of stimulation is too high, and it

recovers after a pause, thus giving rise to the alternation.

The temperature characteristic (μ) ,approximately 7500 cal. calculated from the relation shown in Fig. 7, may be compared with the value 8000 cal. obtained by Hoagland[13] from the relation between α-rhythms of 5 patients and body temperature. He obtained 11000 cal. in other two cases and 16000 cal. in two further cases.

SUMMARY

The behavior of the recruiting response during hypothermia was investigated, and the following results were obtained.

1. Recruiting response was recorded from the frontal area by stimulation of the nucleus ventralis anterior or the anterior pole of the reticular nucleus in rat. The brain temperature was lowered gradually till 18°C. and then raised slowly to normal temperature. All the changes to be stated below were reversible.

2. Spontaneous background EEG was decreased in amplitude, and slowed in rate as the brain temperature was lowered, and flattened at 18°C. Dominant spindle bursts of high amplitude and low frequency appeared between 29°C.–25°C.

3. The optimal frequency of stimulation was determined for each temperature. There was linear relationship between the logarithm of the optimal frequency and the brain temperature in a range from 36°C. to 18°C.

4. A value of temperature characteristic (μ) 7500 cal. was obtained from the law of Arrhenius applied to the data.

5. Large and small responses appeared alternately when the frequency of stimulation was somewhat higher than the optimal one, and only small ones appeared when the frequency was much higher. The mechanism of alternation was discussed.

I wish to thank Prof. K. Motokawa for his invaluable discussion and suggestion throughout the course of the experiment and the preparation of the manuscript.

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Statistical Studies on the Correlation between the ABO-Blood Groups and Some Diseases

By

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In 1921, W. Alexander[1] first pointed out that the blood groups of patients with some diseases show a significant difference in their frequency distribution from those of normal subjects, but in the same year, Buchanan and Higley[2] published a report denying such a correlation. Thus, the problem has come into the attention of the researchers in the field. At first, the correlation of the blood groups with malignant tumors was the main theme of study, but no consensus could be arrived at in the conclusions of different authors and the subject was left in the cold for some time till around 1950 it came again into active study, with the advance in statistical research methods, and especially in England and U.S.A. many new works on the problem were published in 1954 through 1956. In recent years, the field of research has been extended from malignant tumors to diseases of other types in general, as fully described in Roberts' review[3].

The present author mapped out the frequency distribution of the blood groups in 15,744 in-patients accommodated in the national, public and private hospitals in Sendai, relying on the Manual of the International Classification of Diseases, Injuries and Causes of Death[4] for classifying the disease types. This frequency distribution of blood groups in the patients was studied in comparison with the frequency distribution in about 21,000 middle-school pupils in Sendai as surveyed by Akaishi[5] for possible significant differences. The relative incidence as proposed by B. Woolf[6] was also computed, by the method somewhat modified by Haldane[7]. The results of the survey with diseases of which the examined patients numbered not less than 50 were as reported hereunder.

MATERIALS AND METHODS

As data, the records of in-patients at the surgical, gynecological and orthopedics clinics of Tohoku University Hospital, Sendai National Hospital,

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TABLE I. Blood Types in Controls and in Patients

International classification			Total	Blood group			
List no.	Disease			A	B	O	AB
Control		No.	21199	7786	4827	6769	1817
		%	100.0	36.7	22.8	31.9	8.6
001–018	Tuberculosis	No.	1771	605	428	583	155
		%	100.0	*34.2	24.2	32.9	8.8
002.a	Pulmonary tuberculosis	No.	723	253	166	252	52
		%	100.0	35.0	23.0	34.9	7.2
003	Pleural tuberculosis	No.	80	26	17	25	12
		%	100.0	32.5	21.3	31.3	*15.0
011	Tuberculosis of intestines, peritoneum, and mesenteric glands	No.	119	44	36	31	8
		%	100.0	37.0	*30.3	26.1	6.7
011.b	Tuberculosis of peritoneum	No.	90	28	28	27	7
		%	100.0	31.1	*31.1	30.0	7.7
012	Tuberculosis of bones and joints, active or unspecified	No.	186	66	39	60	21
		%	100.0	35.5	21.0	32.3	11.3
012.a	Active or unspecified tuberculosis of vertebral column	No.	72	20	17	24	11
		%	100.0	27.8	23.6	33.3	*15.3
015	Tuberculosis of lymphatic system	No.	69	23	15	26	5
		%	100.0	33.3	21.7	37.7	7.2
016	Tuberculosis of genito-urinary system	No.	578	187	150	184	57
		%	100.0	*32.4	26.0	31.8	9.9
130.0	Ascariasis	No.	74	31	18	20	5
		%	100.0	41.9	24.3	27.0	6.8
140–199	Malignant neoplasms	No.	3921	1512	875	1169	365
		%	100.0	*38.6	22.3	**29.8	9.3
150	Malignant neoplasm of esophagus	No.	157	53	45	42	17
		%	100.0	33.8	28.7	26.8	10.8
151	Malignant neoplasm of stomach	No.	1671	670	358	499	144
		%	100.0	**40.1	21.4	29.9	8.6
153	Malignant neoplasm of large intestine, except rectum	No.	107	34	22	38	13
		%	100.0	31.8	20.6	35.5	12.1
154	Malignant neoplasm of rectum	No.	224	88	56	62	18
		%	100.0	39.3	25.0	27.7	8.0

		%	100.0	39.3	25.0	27.7	8.0
155	Malignant neoplasm of biliary passages and of liver (stated to be primary site)	No.	66	21	17	19	9
		%	100.0	31.8	25.8	28.8	13.6
157	Malignant neoplasm of pancreas	No.	65	29	11	20	5
		%	100.0	44.6	16.9	30.8	7.7
170	Malignant neoplasm of breast	No.	167	56	34	59	18
		%	100.0	33.5	20.4	35.3	10.8
171	Malignant neoplasm of cervix uteri	No.	832	318	181	255	78
		%	100.0	38.2	21.8	30.6	9.4
194	Malignant neoplasm of thyroid gland	No.	54	24	13	11	6
		%	100.0	44.4	24.1	20.4	11.1
196	Malignant neoplasm of bone (including jaw bone)	No.	89	41	19	23	6
		%	100.0	46.1	21.3	25.8	6.7
200	Lymphosarcoma and reticulosarcoma	No.	50	17	18	12	3
		%	100.0	34.0	*36.0	24.0	6.0
210–229	Benign neoplasm	No.	1149	438	264	358	89
		%	100.0	38.1	23.0	31.2	7.7
211	Benign neoplasm of other parts of digestive system	No.	67	25	16	22	4
		%	100.0	37.3	23.9	32.8	6.0
214	Uterine fibromyoma	No.	424	162	94	129	39
		%	100.0	38.2	22.2	30.4	9.2
215	Other benign neoplasm of uterus	No.	51	20	10	13	8
		%	100.0	39.2	19.6	25.5	15.7
216	Benign neoplasm of ovary	No.	428	170	97	137	24
		%	100.0	39.7	22.7	32.0	*5.6
237	Neoplasm of unspecified nature of brain and other parts of nervous system	No.	97	35	22	34	6
		%	100.0	36.1	22.7	35.1	6.2
241	Asthma	No.	78	26	24	22	6
		%	100.0	33.3	30.8	28.2	7.7
251	Non-toxic nodular goitre	No.	102	38	23	31	10
		%	100.0	37.3	22.5	30.1	9.8
252	Thyrotoxicosis with or without goitre	No.	214	75	53	70	16
		%	100.0	35.0	24.8	32.7	7.5
353	Epilepsy	No.	86	33	23	22	8
		%	100.0	38.4	26.7	25.6	9.3
461	Haemorrhoids	No.	177	59	31	75	12
		%	100.0	33.3	17.5	**42.4	6.8

* Significance at 5% level ** Significance at 1% level

TABLE I. continued

International classification			Total	Blood group			
List no.	Disease			A	B	O	AB
540	Ulcer of stomach	No.	511	158	112	195	46
		%	100.0	**30.9	21.9	**38.2	9.0
541	Ulcer of duodenum	No.	333	101	88	112	32
		%	100.0	**30.3	26.4	33.6	9.6
550	Acute appendicitis	No.	887	314	191	303	79
		%	100.0	35.4	21.5	34.2	8.9
560	Hernia of abdominal cavity without mention of obstruction	No.	169	62	38	54	15
		%	100.0	36.7	22.5	32.0	8.9
570	Intestinal obstruction without mention of hernia	No.	262	104	68	67	23
		%	100.0	39.7	26.0	*25.6	8.8
574	Anal fissure and fistula	No.	107	46	16	30	15
		%	100.0	43.0	*15.0	28.0	*14.0
576	Peritonitis	No.	61	21	13	22	5
		%	100.0	34.4	21.3	36.1	8.2
578	Other diseases of intestines and peritoneum	No.	121	52	12	45	12
		%	100.0	43.0	**9.9	37.2	9.9
584, 585	Cholelithiasis and cholecystitis	No.	351	120	83	106	42
		%	100.0	34.2	23.6	30.2	*12.0
584	Cholelithiasis	No.	234	80	51	79	24
		%	100.0	34.2	21.8	33.8	10.3
585	Cholecystitis without mention of caculi	No.	117	40	32	27	18
		%	100.0	34.2	27.4	*23.1	*15.4
586	Other diseases of gallbladder and biliary ducts	No.	69	31	12	21	5
		%	100.0	44.9	17.4	30.4	7.2
587	Diseases of pancreas	No.	50	16	16	12	6
		%	100.0	32.0	32.0	24.0	12.0
592	Chronic nephritis	No.	72	20	22	26	4
		%	100.0	27.8	30.6	36.1	3.6
600	Infections of kidney	No.	59	26	11	20	2
		%	100.0	44.1	18.6	33.9	3.4
602	Calculi of kidney and ureter	No.	131	49	22	50	10
		%	100.0	37.4	16.8	38.2	7.6

611–617	Diseases of male genital system (excluding hyperplasia of prostate)	No.	49	23	8	15	3
		%	100.0	46.9	16.3	30.6	6.1
620, 621	Diseases of breast	No.	153	64	38	36	15
		%	100.0	41.8	24.8	*23.5	9.8
620	Chronic cystic disease of breast	No.	116	51	26	27	12
		%	100.0	44.0	22.4	*23.3	10.3
622–637	Diseases of female genital system	No.	975	345	223	310	97
		%	100.0	35.4	22.9	31.8	9.9
622	Acute salpingitis and oophoritis	No.	71	28	17	23	3
		%	100.0	39.4	23.9	32.4	4.2
623	Chronic salpingitis and oophoritis	No.	63	22	16	18	7
		%	100.0	34.9	25.4	28.6	11.1
624	Salpingitis and oophoritis, unqualified	No.	50	16	15	16	3
		%	100.0	32.0	30.0	32.0	6.0
625	Other diseases of ovary and Fallopian tube	No.	69	25	16	26	2
		%	100.0	36.2	23.2	37.7	2.9
626	Diseases of parametrium and pelvic peritoneum (female)	No.	156	49	33	57	17
		%	100.0	31.4	21.2	36.5	10.9
630	Infective disease of uterus, vagina, and vulva	No.	131	50	29	39	13
		%	100.0	38.2	22.1	29.8	9.9
631	Uterovaginal prolapse	No.	72	30	14	22	6
		%	100.0	41.7	19.4	30.6	8.3
632	Malposition of uterus	No.	210	77	50	59	24
		%	100.0	36.7	23.8	28.1	11.4
634	Disorders of menstruation	No.	87	31	17	28	11
		%	100.0	35.6	19.5	32.2	12.6
645	Ectopic pregnancy	No.	113	41	35	24	13
		%	100.0	36.3	*31.0	*21.2	11.5
648	Other complications arising from pregnancy	No.	61	21	17	20	3
		%	100.0	34.4	27.9	32.8	4.9
650	Abortion without mention of sepsis or toxaemia	No.	61	17	17	23	4
		%	100.0	27.9	27.9	37.7	6.6
642, 652, 685, 686	Toxaemias of pregnancy and the puerperium	No.	79	27	16	30	6
		%	100.0	34.2	20.2	38.0	6.6
643, 644, 670–672	Haemorrhage of pregnancy and childbirth	No.	85	31	17	24	13
		%	100.0	36.5	20.0	28.2	*15.3
672	Delivery complicated by other postpartum haemorrhage	No.	54	16	13	15	10
		%	100.0	29.6	24.1	27.8	*18.5

TABLE I continued

International classification			Total	Blood group			
List no.	Disease			A	B	O	AB
645–649, 673–680, 683, 687–689	Other complications of pregnancy, childbirth and the puerperium	No.	254	97	63	72	22
		%	100.0	38.2	24.8	28.3	8.7
730	Osteomyelitis and periostitis	No.	123	50	26	37	10
		%	100.0	40.7	21.1	30.1	8.1
735	Displacement of intervertebral disc	No.	72	18	23	22	9
		%	100.0	*25.0	*31.9	30.6	12.5
738	Other diseases of joint	No.	55	25	13	14	3
		%	100.0	45.5	23.6	25.5	5.5
750–759	Congenital malformations	No.	491	184	96	161	50
		%	100.0	37.5	19.6	32.8	10.2
756	Congenital malformations of digestive system	No.	249	93	40	93	23
		%	100.0	37.3	**16.1	37.3	9.2
757	Congenital malformations of genito-urinary system	No.	71	20	18	23	10
		%	100.0	28.2	25.4	32.4	14.1
758	Congenital malformations of bone and joint	No.	117	51	26	28	12
		%	100.0	43.9	22.2	23.9	10.1
N800–N999	Accidents, poisonings, and violence	No.	643	237	142	224	40
		%	100.0	36.9	22.1	34.8	*6.2

Sendai Municipal Hospital, Sendai Red-Cross Hospital, Tohoku Labor-Casualty Hospital, Geiko-en Sanatorium and Katta County Hospital, bearing the individual blood groups and confirmation of the disease by surgical operation or X-ray examination were used. Of cases with complicating diseases, the cases with mutually unrelated diseases such as pulmonary tuberculosis and acute appendicitis were counted in the groups of all the individual diseases.

The normal frequency distribution, as determined by Akaishi[5] in 21,199 middle-school pupils in Sendai, and used as control in this study, was shown in Table I.

For detecting significant differences between the rates of blood groups in patients of a disease and the rates in controls, the 2×2 table was used and the level of significance was set 5% ($\chi^2=3.841$).

At computing the relative incidence, the incidence was taken as significant in the cases when the hypothesis: relative incidence = 1 is to be rejected at 5% level of significance.

RESULTS

Of the groups of more than 50 patients per disease, the actual numbers and the percentages of the cases with different blood groups are shown in Table I and the relative incidences of these diseases computed by these data are given in Table II. The items showing significant differences from the control frequency are as described in the following.

1. The frequency of Group A was found significantly lower among the tuberculous cases of all sites than among the controls, but of the other groups, no such difference could be observed. Trorbridge[8] surveyed the frequency distribution in 1,400 tuberculous cases and was led to deny any correlation. Upon computation of the relative incidence, it was found that the frequency of tuberculosis occurring in Group A cases was significantly higher than that in Group B cases.

By sites of the tuberculosis, the percentage of Group AB was significantly high in pleural tuberculosis and active or unspecified tuberculosis of vertebral column, Group A was significantly frequent in tuberculosis of genito-urinary system and Group B in tuberculosis of peritoneum. Akita[9] has pointed out that Group A cases were low and Group B high in frequency among patients with pulmonary tuberculosis, and Group B cases were not frequent among pleural tuberculosis cases, but according to my survey, there was no significant difference in the frequency of any blood group in pulmonary tuberculosis cases from that in the controls, while among the pleural tuberculosis cases the frequency of Group AB was significantly higher than the control. The computed relative incidence showed that the frequency of pleural or vertebral tuberculosis occurring among Group A cases was lower than in Group AB cases, and that of tuberculosis of

TABLE II. Relative Incidence

International classification		Relative incidence					
List no.	Disease	A : B	A : O	A : AB	B : O	B : AB	O : AB
001–018	Tuberculosis	*0.88	0.90	0.90	1.03	1.04	1.01
002.a	Pulmonary tuberculosis	0.94	0.87	1.13	0.92	1.19	1.29
003	Pleural tuberculosis	0.94	0.90	*0.49	0.96	0.53	0.55
011	Tuberculosis of intestines, peritoneum, and mesenteric glands	0.76	1.23	1.22	*1.62	1.62	0.99
011.b	Tuberculosis of peritoneum	0.62	0.90	0.89	1.45	1.43	0.98
012	Tuberculosis of bones and joints, active or unspecified	1.04	0.96	0.72	0.92	0.69	0.76
012.a	Active or unspecified tuberculosis of vertebral column	0.73	0.72	*0.42	1.00	0.57	0.57
015	Tuberculosis of lymphatic system	0.94	0.77	1.00	0.82	1.06	1.29
016	Tuberculosis of genito-urinary system	*0.77	0.88	0.76	1.14	0.99	0.86
130.0	Ascariasis	1.06	1.34	1.34	1.27	1.27	1.00
140–199	Malignant neoplasms	1.07	**1.12	0.97	1.05	0.90	*0.86
150	Malignant neoplasm of esophagus	0.73	1.09	0.71	1.50	0.98	0.65
151	Malignant neoplasm of stomach	*1.16	**1.17	1.08	1.01	0.67	0.93
153	Malignant neoplasm of large intestine, except rectum	0.95	0.78	0.60	0.82	0.63	**0.77
154	Malignant neoplasm of rectum	0.97	1.23	1.12	1.27	1.15	0.91
155	Malignant neoplasm of biliary passages and of liver (stated to be primary site)	0.76	0.96	0.53	1.26	0.69	0.55
157	Malignant neoplasm of pancreas	1.65	1.25	1.25	0.79	0.79	1.00
170	Malignant neoplasm of breast	1.02	0.83	0.71	0.81	0.70	0.86
171	Malignant neoplasm of cervix uteri	1.09	1.08	0.95	1.00	0.87	0.87
194	Malignant neoplasm of thyroid gland	1.13	1.85	0.88	1.65	0.78	0.48
196	Malignant neoplasm of bone (including jaw bone)	1.32	1.54	1.49	1.16	1.13	0.97
200	Lymphosarcoma and reticulosarcoma	0.59	1.22	1.17	*2.08	1.99	0.96
210–229	Benign neoplasm	1.03	1.06	1.14	1.03	1.12	1.08
211	Benign neoplasm of other parts of digestive system	0.96	0.99	1.32	1.03	1.38	1.34

* Significance at 5% level ** Significance at 1% level

*Significance at 5% level **Significance at 1% level

TABLE II continued

International classification		Relative incidence					
List no.	Disease	A : B	A : O	A : AB	B : O	B : AB	O : AB
214	Uterine fibromyoma	1.07	1.09	0.96	1.02	0.90	0.88
215	Other benign neoplasm of uterus	1.21	1.32	0.56	1.09	0.47	*0.43
216	Benign neoplasm of ovary	1.08	1.08	*1.62	0.99	1.47	1.51
237	Neoplasm of unspecified nature of brain and other parts of nervous system	0.98	0.89	1.27	0.91	1.30	1.43
241	Asthma	0.67	1.02	0.95	1.53	1.39	0.93
251	Non-toxic nodular goitre	1.02	1.06	0.86	1.05	0.84	0.81
252	Thyrotoxicosis with or without goitre	0.87	0.93	1.07	1.06	1.20	1.15
353	Epilepsy	0.88	1.29	0.92	1.46	1.04	0.71
461	Haemorrhoids	1.19	*0.69	1.11	*0.59	0.95	1.62
540	Ulcer of stomach	0.87	**0.70	0.80	0.81	0.91	1.13
541	Ulcer of duodenum	*0.71	0.78	0.73	1.10	1.03	0.93
550	Acute appendicitis	1.02	0.90	0.92	0.88	0.91	1.02
560	Hernia of abdominal cavity without mention of obstruction	1.01	1.00	0.94	0.99	0.94	0.94
570	Intestinal obstruction without mention of hernia	0.95	1.35	1.04	*1.42	1.10	0.77
574	Anal fissure and fistula	*1.75	1.33	0.70	0.76	**0.40	*0.53
576	Peritonitis	0.99	0.83	0.91	0.84	0.92	1.10
578	Other diseases of intestines and peritoneum	**2.60	1.00	0.98	**0.39	**0.38	0.98
584, 585	Cholelithiasis and cholecystitis	0.89	0.98	*0.66	1.10	0.74	*0.67
584	Cholelithiasis	0.97	0.88	0.77	0.91	0.79	0.87
585	Cholecystitis without mention of calculi	0.77	1.28	*0.51	*1.66	0.66	**0.40
586	Other diseases of gallbladder and biliary ducts	1.56	1.27	1.34	0.82	0.86	1.05
587	Diseases of pancreas	0.62	1.15	0.59	1.85	0.96	0.52
592	Chronic nephritis	0.56	0.67	1.06	1.19	1.88	1.58
600	Infections of kidney	1.43	1.12	2.47	0.79	1.73	2.20
602	Calculi of kidney and ureter	1.36	0.85	1.10	0.62	0.81	1.29
611–617	Diseases of male genital system (excluding hyperplasia of prostate)	1.71	1.32	1.57	0.77	0.91	1.19

TABLE II continued

International classification		Relative incidence					
List no.	Disease	A : B	A : O	A : AB	B : O	B : AB	O : AB
620, 621	Diseases of breast	1.04	*1.54	0.97	1.48	0.94	0.63
620	Chronic cystic disease of breast	1.20	*1.63	0.96	1.35	0.80	0.59
622–637	Diseases of female genital system	0.96	0.97	0.83	1.01	0.86	0.86
622	Acute salpingitis and oophoritis	1.01	1.05	1.90	1.04	1.88	1.80
623	Chronic salpingitis and oophoritis	0.85	1.06	0.70	1.25	0.83	0.66
624	Salpingitis and oophoritis, unqualified	0.66	0.87	1.10	1.32	1.67	1.27
625	Other diseases of ovary and Fallopian tube	0.96	0.84	2.38	0.87	2.48	2.85
626	Diseases of parametrium and pelvic peritoneum (female)	0.92	0.75	0.66	0.82	0.72	0.88
630	Infective disease of uterus, vagina, and vulva	1.06	1.11	0.87	1.05	0.82	0.79
631	Uterovaginal prolapse	1.30	1.18	1.10	0.90	0.84	0.93
632	Malposition of uterus	0.95	1.13	0.74	1.19	0.78	0.65
634	Disorders of menstruation	1.12	0.96	0.64	0.86	0.57	0.67
645	Ectopic pregnancy	0.72	1.47	0.72	**2.03	0.99	*0.49
648	Other complications arising from pregnancy	0.76	0.91	1.43	1.20	1.88	1.57
650	Abortion without mention of sepsis or toxaemia	0.62	0.65	0.91	1.04	1.46	1.40
642, 652, 685, 686	Toxaemias of pregnancy and childbirth	1.03	0.78	0.99	0.76	0.96	1.26
643, 644, 670–672	Haemorrhage of pregnancy and childbirth	1.12	1.12	0.54	1.00	*0.49	*0.49
672	Delivery complicated by other postpartum haemorrhage	0.76	0.93	**0.37	1.22	0.49	*0.40
645–649, 673–680, 683, 687–689	Other complications of pregnancy, childbirth and the puerperium	0.95	1.17	1.01	1.23	1.06	0.87
730	Osteomyelitis and periostitis	1.18	1.17	1.12	0.99	0.95	0.96
735	Displacement of intervertebral disc	*0.49	0.71	*0.45	1.46	0.93	0.64
738	Other diseases of joint	1.17	1.52	1.70	1.31	1.45	1.11
750–759	Congenital malformations	1.19	0.99	0.85	0.84	0.72	0.86
756	Congenital malformations of digestive system	1.43	0.87	0.93	**0.61	0.65	1.07

International classification		Relative incidence					
List no.	Disease	A : B	A : O	A : AB	B : O	B : AB	O : AB
757	Congenital malformations of genito-urinary system	0.69	0.76	*0.46	1.10	0.66	0.60
758	Congenital malformations of bone and joint	1.20	1.57	0.96	1.30	0.79	0.61
N800–N999	Accidents, poisonings, and violence	1.03	0.92	1.37	0.89	1.32	*1.49

TABLE III. Extract of Table I Showing Significant Differences Only

International Classification		Number of total cases	Blood group			
List no.	Disease		A	B	O	AB
001–018	Tuberculosis	1771	(-)			
003	Pleural tuberculosis	80				(+)
011	Tuberculosis of intestines, peritoneum, and mesenteric glands	119		(+)		
011.b	Tuberculosis of peritoneum	90		(+)		(+)
012.a	Active of unspecified tuberculosis of vertebral column	72				
016	Tuberculosis of genito-urinary system	578	(-)			
140–199	Malignant neoplasms	3921	(+)		(--)	
151	Malignant noplasm of stomach	1671	(++)			
200	Lymphosarcoma and reticulosarcoma	50		(+)		
216	Benign neoplasm of ovary	428				(-)
461	Haemorrhoids	177			(++)	
540	Ulcer of stomach	511	(--)		(++)	
541	Ulcer of duodenum	333	(--)			
570	Intestinal obstruction without mention of hernia	262			(-)	
574	Anal fissure and fistula	107		(-)		(+)
578	Other diseases of intestines and peritoneum	121		(--)		

TABLE III continued

International Classification		Number of total cases	Blood group			
List no.	Disease		A	B	O	AB
584, 585	Cholelithiasis and cholecystitis	351				(+)
585	Cholecystitis without mention of calculi	117			(--)	(+)
620, 621	Diseases of breast	153			(−)	
620	Chronic cystic disease of breast	116			(−)	
645	Ectopic pregnancy	113		(+)	(−)	
643, 644, 670–672	Haemorrhage of pregnancy and childbirth	85				(+)
672	Delivery complicated by other postpartum haemorrhage	54				(+)
735	Displacement of intervertebral disc	72	(−)	(+)		
756	Congenital malformations of digestive system	249		(--)		
N800–N999	Accidents, poisonings, and violence	643				(−)

(++) Higher at 1% level of significance
(+) Higher at 5% level of significance
(--) Lower at 1% level of significance
(−) Lower at 5% level of significance

genito-urinary system in Group A was lower than in Group B cases.

2. In the cases of malignant neoplasm (not including those of the lymphatic tissue and hematopoietic system), the frequency of Group A was significantly high and that of Group 0 was low. Since W. Alexander[1] first pointed out such a correlation, many reports have been given out on the subject. On the one hand, Weitzner & Schiff[10], Johansen[11], Flamm & Friedlich[12] and Terada[13] concluded in affirmative on the correlation between malignant tumors and blood groups, while on the other hand, Hoche & Moritsch[14], Nakajima *et al.*[15], Inada[16], Chang[17], Shimizu *et al.*[18] and Walther *et al.*[19] denied such a correlation. The frequency of malignant neoplasms occurring in Group A and Group AB cases is higher than in Group O cases.

In the cases with malignant neoplasm of the stomach, the frequency of Group A cases was significantly higher than the control, and the frequency of incidence to stomach cancer was higher in Group A than in Group B or Group O cases, and lower in Group B than in Group AB cases. Aird *et al.*[20], Kster *et al.*[21], Buckwalter *et al.*[22], Segi *et al.*[23], and Noie *et al.*[24] report that the percentage of Group A cases is supernormal in stomach cancer cases, but Inada[16] denied any such deviation. No significant difference from the normal was found in any blood group in the cases with malignant neoplasms of large intestine and rectum, but the relative incidence revealed that malignant neoplasms of large intestine except rectum showed lower incidence in Group 0 cases than in Group AB cases. Aird *et al.*[20], Buckwalter *et al.*[22], Walther *et al.*[19] and Mcconnel *et al.*[25] have also arrived at negative results in their search for possible correlation between the blood groups and malignant neoplasms in these parts.

3. In lymphosarcoma and reticulosarcoma cases, the frequency of Group B was found above normal and the incidence to these neoplasms was significantly higher in Group B cases than in Group O cases.

4. In the cases with benign neoplasms of all sites, no significant difference from the normal was found in the frequency of any blood group, but among the cases with such neoplasms in the ovary, the Group AB showed significantly subnormal frequency and the incidence to such ovarian tumors was found higher in the Group A cases than in Group AB cases.

5. In the cases with haemorrhoids the rate of Group 0 cases was above normal and the incidence to this blood group of disease was higher in Group 0 cases than in Group A or Group B cases.

6. In the cases with ulcer of stomach, the frequency of Group A was lower and that of Group 0 cases higher than the control. The incidence to such ulcers was higher among the Group 0 than among the Group A cases. J.A. Buckwalter *et al.*[22] and Kurokawa-Masuda *et al.*[26] also point out the supernormal frequency of Group 0 cases among the stomach ulcer cases, but Akita[9] has refused to admit such a deviation from the normal in the frequency of any blood group in this

disease.

7. In the cases with ulcer of duodenum, Group A was less than normally frequent and the relative incidence to this disease was lower in Group A than in Group B cases.

8. In the cases with intestinal obstruction without mention of hernia, the rate of Group 0 was subnormally low and the incidence in the Group 0 cases was also lower than in the Group B cases.

9. In the cases with anal fissure and fistula, the frequency of Group B was lower and that of AB was higher than normal. The incidence to this blood group of diseases was higher in Group A than in Group B cases and lower in Group B and Group 0 cases than in Group AB cases.

10. In the cases of other diseases of intestine and peritoneum, the frequency of Group B cases was subnormal, while the incidence in Group B cases is lower than in cases of all the other blood groups.

11. In the cases with cholelithiasis and cholecystitis, the frequency of Group AB cases was supernormally high and the incidence to these diseases in these cases was higher than in Group A or Group 0 cases. In the cases of cholecystitis in exclusion of those with mentioned caliculi, the Group 0 cases were less and Group AB cases more frequent than the control. The incidence to these diseases in Group AB cases is higher than in Group A or Group 0 cases and higher in Group B cases than in Group 0 cases.

12. In the cases with diseases of breast, Group 0 cases were less frequent than the control and the incidence in these cases was also lower than in Group A cases. Similar results were obtained when the frequency distribution and the relative incidence among the cases with chronic cystic diseases of breast alone were studied.

13. In ectopic pregnancy cases, the frequency of Group B cases was higher and that of Group 0 cases lower than normal. The incidence to this anomaly was higher in Group B cases and Group AB cases than in Group 0 cases.

14. In the cases of haemorrhage of pregnancy and childbirth, the frequency of Group AB cases was supernormal and the incidence among them was also higher than in Group B or Group 0 cases. The same may be said of the cases falling under the subitem of delivery complicated by postparum haemorrhage mainly of atonic bleeding.

15. In the cases with displacement of intervertebral disc, the frequency of Group A cases was lower and that of Group B cases higher than normal. The incidence in Group A cases was lower than that in Group B and AB cases.

16. In the cases with congenital malformation of all types and sites, no blood group showed a mean frequency significantly deviating from that the control, but when we limit ourselves to such malformations of the digestive and the urogenital organs, in the cases with the former the frequency of Group B cases was

significantly subnormal and the incidence in these cases was lower than in Group 0 cases, while in the cases with the latter malformations the incidence was higher in Group A than in Group AB cases.

17. In the cases injured by accidents, poisoning and violence, the frequency of Group AB cases was lower than normal and the relative incidence was also lower in these than in Group 0 cases.

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Histochemical Studies on the Metabolism of Fat and Vitamin A of Placenta

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Of the authors who have reported on the metabolism of fat and vitamin A through the placenta playing such an important part in the growth of the fetus, Effkemann[1] says that fat is formed in the fetal body either from the fractions of fat that have been transported into it or from glucides and protein fractions that have passed through the placenta, and more recently it has been claimed that the chorionic epithelium has the function of ingesting fatty acids and lipoids demanded by the fetus from the maternal side and supplying them to the fetus[2][3]; lately, Goldwater[4] and Popjak[5] experimented with isotopes and demonstrated that the fat in the fetus is mainly elaborated in the fetus. Dann[6] found vitamin A is permeable through the placenta of rats and rabbits, and Gaehtgens[7] reported that the placenta conserves vitamin A and carotene and that both these substances are transported to the fetal side in the form of carotene to be stored in the fetal liver and to form vitamin A on demand; Barnes[8] made follow-up experiments and confirmed this proposition. Stegmann[9] also admits the transition of vitamin A through the placenta, and from the finding that the concentration of vitamin A in the maternal and the fetal side does not change proportionately, opines that the placenta may have the function of controlling the vitamin A transition. All the above reports have been based on biochemical studies and none has ever studied the histochemical process of fat and vitamin A through the chorionic epithelium, as the present author reports in the following.

MATERIALS AND METHODS

As experimental materials, human placentae in various stages of normal pregnancy and rats in the last stage of gestation were used. The substances used in this study were as follows. Fatgen, a fat emulsion made of purified sesame oil, was used as source of fat and water-soluble Chocola A and water-soluble and oil-soluble carotene specially prepared for the experiments as the source of vitamin A.

奥田宜弘

The method of the experiments was as follows:

1) Fat and Vitamin A Administration from the Maternal Side: Rats in the last stage of gestation were injected with 10 g./kg. of Fatgen, 250,000 units/kg. of Chocola A or 10 mg/kg. of carotene through the femoral vein without anesthetization. Vitamin A and carotene were given to such rats also orally or intramuscularly. After administration, the animals were sacrificed at various intervals and their placenta and the liver of the mother and the youngs were excised for examination. On the other hand, the pregnant women were intravenously injected with 50 g. of Fatgen or 300,000 units of Chocola A each 30 minutes before artificial abortion or delivery and those placentae were examined. For staining fat, sudan III was used, the Gomori's method for lipase, the Okamoto's method for fatty acids and for vitamin A the fluoromicroscopic method with glyceroldichlorhydrin method to supplement it.

For determining the local existence of vitamin A, haematoxylin-eosin staining and sudan III staining were used to supplement the fluoromicroscopic method. For preparing thin sections of the placenta, the current process was partly modified, namely, the materials were fixed in 20% formalin solution for 2 hours, rinsed in water for about 30 minutes and then embedded in gelatin before cutting into sections. The fluorescence from gelatin could be easily differentiated from that from vitamin A.

2) Incubation: Placentae from subjects in the first and the last stages of gestation were incubated in isotonic solution of Fatgen, Chocola A or carotene at 37°C for 15–60 minutes under presence of abundant O_2 and their fat and vitamin A contents were measured.

3) Perfusion: Placentae sampled immediately after delivery were perfused with similar solutions through the umbilical artery and their fat and vitamin A contents were measured at stated intervals.

RESULTS

(I) Fat

1) Fat Administration: As shown in Fig. 1, 15 minutes after Fatgen administration, fine droplets of migrating fat were seen in the blood capillaries in the labyrinthine layer of the fetus side of the placenta of rats. The fat content of the syncytium cells was not changed, but that in the decidual cells and the yolk sac increased with lapse of time (Fig. 2). Lipase and fatty acids were found negative in the fetal liver too, fat was found beginning to increase 15 minutes after the administration (Fig. 3). When fat was administered to human gravidae, no significant difference was found in the result from that of normal subjects.

2) Incubation in Fat Solution: After incubation in 1% Fatgen solution,

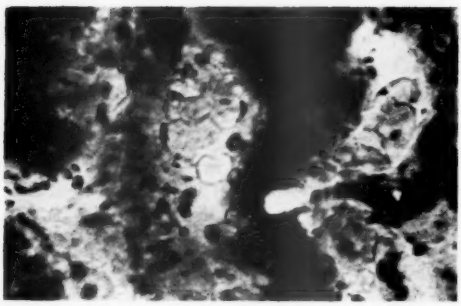


Fig. 1. Placenta of rat, 15 minutes after administration of large amount of Fatgen. The fat granules transferred into the fetal capillaries are found. (sudan III, ×600).

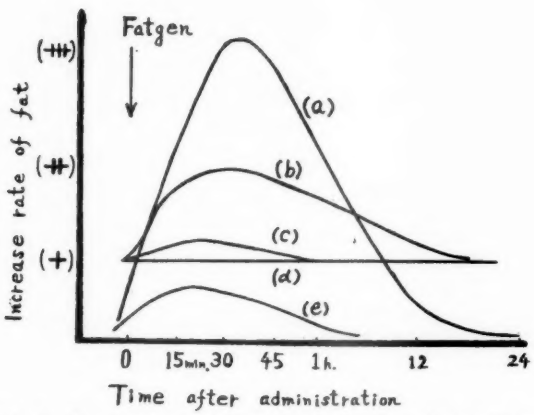
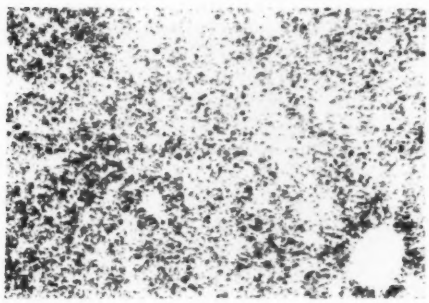


Fig. 2. Fat in the rat placenta after Fatgen administration.
(a): Maternal blood vessel, (b): Decidua,
(c): Yolk sac, (d): Syncytium, (e): Fetal capillary

fat granules were found adhering to the chorionic epithelium of placentae of early as well as late stage of gestation. When 25% of bovine bile was added to the Fatgen solution, fat granules were found as if detained in the basement membrane beneath the syncytium cells after 30 minutes of incubation (Fig. 4: Table I).

3) Perfusion with Fatgen Solution: After 30 minutes of perfusion with isotonic Fatgen solution mixed with 25% of bovine bile, fat granules were found having reached the basement membrane via blood capillaries (Fig. 5). For

a



b

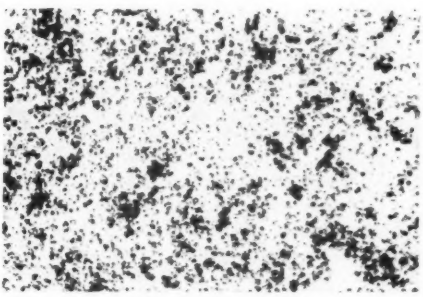


Fig. 3. Control liver of rat fetus (a). Liver of rat fetus, 15 minutes after injection of Fatgen to the mother rat (b). (sudan III, ×400)

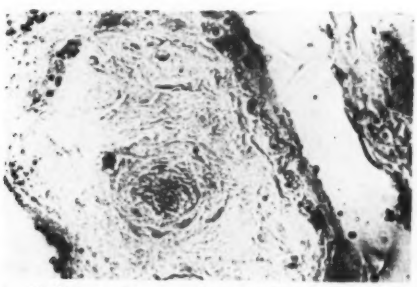


Fig. 4. Full term human placenta, incubated in Fatgen with bile for one hour. Fat granules are found on the surface of the syncytium cells and in linear arrangement beneath the syncytium cells. (sudan III, ×400)

Table I. Fat Content after Incubation

	Time of Incubation		Controls	15 min.	30 min.	1 hour
Fat emulsion puls bovine bile	Normal placenta	Surface	− (−)	++ (++)	++ (++)	++ (++)
		Syncytium	−~± (+)	−~± (+)	−~± (+~++)	−~± (+~++)
		beneath Sy. (Basement Memb.)	− (−)	− (−)	± (±)	± (±)
		Langhans	(−)	(−)	(−)	(−)
		Stroma	−~± (−~±)	−~± (−~±)	−~± (−~±)	± (±)

Note: Figures in parenthese are for placenta of the early stage of gestation

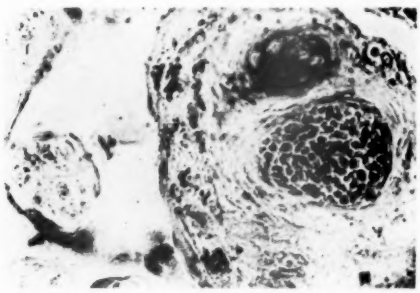


Fig. 5. Full term human placenta, perfused through the umbilical artery with Fatgen-bile solution for one hour. Fat granules are found arranged in lines in the basal part of the syncytium cells. (sudan III, ×400)

determining whether the bovine bile affects the permeability through the chorionic epithelium and the capillary walls, bovine bile was added to Indian ink and similar perfusion was carried out, but the permeability was found unchanged.

(II) Vitamin A

1) Vitamin A and Carotene Administration: As shown in Fig. 6, vitamin A began to appear in the blood vessels of the maternal side of the placenta soon after intravenous administration of Chocola A and reached the maximum concentration in 30 minutes. In the control placentae, vitamin A was found negative in the syncytium cells but after vitamin A administration, vitamin A began to come forth in about 30 minutes. Increase of vitamin A was observed also in the

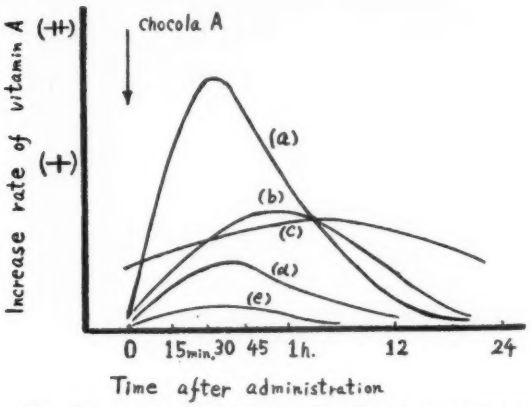


Fig. 6. Vitamin A in the rat placenta after Chocola A administration.
(a) : Maternal blood vessel, (b) : Decidua, (c) : Yolk sac,
(d) : Syncytium, (e) : Fetal capillary

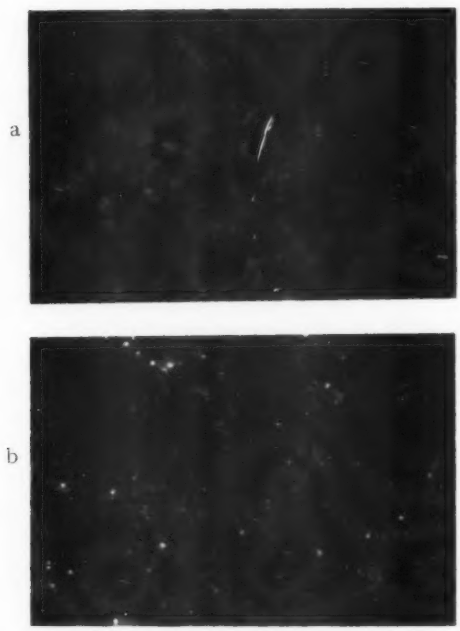


Fig. 7. Control liver of rat fetus, vitamin A is negative (a). Liver of rat fetus, one hour after administration of large amount of Chocola A. Vitamin A become positive (b). (Fluoromicroscopic method, ×400)

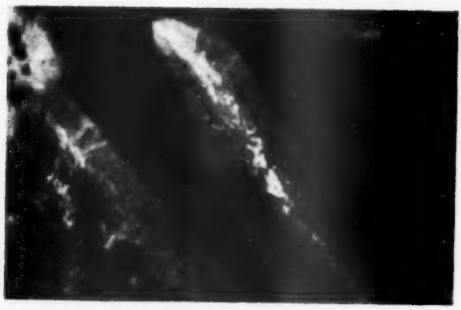


Fig. 8. Small intestine of rat, one hour after Chocola A administration per os. (Fluoromicroscopic method, ×400)

Fig. 9. Human placenta from 12 weeks pregnancy, incubated in Chocola A for 30 minutes. Fluorescence due to vitamin A is most intense on the surface of the syncytium cells and in the cells themselves and it was found in the intercellular spaces of the Langhans cells. (Fluoromicroscopic method, ×400)

decidual cells and the fluorescence due to vitamin A in the yolk sac was intensified. Vitamin A was found increased in the maternal liver too, and in the fetal liver it became positive one hour after the administration to the mother rats (Fig. 7). Vitamin A was lacking in the yolk sac after feeding two weeks with vitamin A-deficient diet[10], but upon administration of vitamin A, the yolk sac became again positive in vitamin A. In the animals given vitamin A per os, the appearance of vitamin A was somewhat retarded, vitamin A being first detected 24 hours after the administration in the fetal liver. Frank absorption of vitamin A was apparent in the small intestine (Fig. 8). Carotene emits perceptible fluorescence only at high concentration, so that after injection of watersoluble carotene by which very high concentration is difficult its presence in the placenta

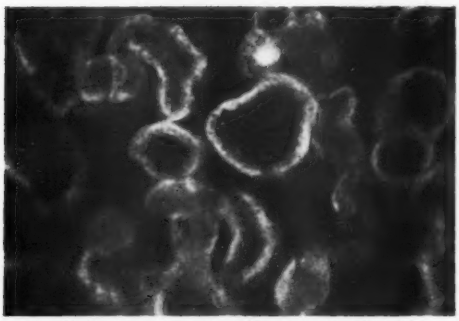


Fig. 10. Full term human placenta, incubated in Chocola A for 30 minutes. Fluorescence due to vitamin A appeares in the syncytium cell cytoplasma and in the basal part of the syncytium cells. (Fluoromicroscopic method, ×600)

TABLE II. Vitamin A Content after Incubation

	Time of Incubation		Controls	15 min.	30 min.	1 hour
Chocola A isotonic solution	Normal placenta	Surface	− (−)	++ (++)	++ (++)	++ (++)
		Syncytium	−~± (±)	±~+ (±~+)	+~++ (+~++)	+~++ (++)
		beneath Sy. (Basement Memb.)	− (−)	± (±)	+ (+)	+ (+)
		Langhans	(−)	(−)	(−)	(−)
		Stroma	− (−)	− (−)	− (−)	−~± (−~±)

Note: Figures in parentheses are for placenta of the early stage of gestation

and the liver could not be traced by fluorescence, except in the case given a large dosis of carotene by oft-repeated injection, wherein, fluorescence due to vitamin A was visible and carotene was also weakly positive in 24 hours after the last injection. When Chocola A was administered to human gravidae, no significant difference was found in the result from that of normal subjects.

2) Incubation in Vitamin A and Carotene Solution: In the placentae of early and late stages of gestation sampled from the controls, vitamin A could be traced in small quantity in the syncytium cells, a little less sparingly in those of the earlier placenta. After incubation in isotonic solution of Chocola A (500,000 units/100 cc), fluorescence due to vitamin A was most intense on the surface of the syncytial cells and next in the cells themselves. In the placenta of early

stage of gestation, the Langhans cells emitted no vitamin A fluorescence; the fluorescence appeared on faces running between the Langhans cells down to the basement membrane in the placenta of early gestation (Fig. 9) and in the mature placenta, in the basement membrane beneath the syncytium cells (Fig. 10, Table II). After incubation in water-soluble carotene, no new fluorescence due to vitamin A could be detected. so that there was no evidence of carotene having changed into vitamin A.

3) Perfusion with Vitamin A and Carotene : Vitamin A was found positive in the blood capillaries of the chorionic membrane and small quantity of vitamin A was detected detained in the basement membrane. After perfusion with carotene solution, carotene was detected in the blood vessels but nowhere else.

DISCUSSION

After administration of large doses of fat and vitamin A to rats in the last stage of gestation, these alimentary substances were found augmented in the liver of the fetus too, histochemically proving the passage of fat and vitamin A through the placenta. The fact that, however, the content of these substances in the fetal liver does not come up to the maximum concentration simultaneously in the blood vessels of the maternal side of the placenta suggests that there is a placental function in controlling the transition of these substances. In the fetal liver, the fat content began to increase 15 minutes after the administration of fat to the mother, but the vitamin A content began to rise after the lapse of one hour after the administration. This discrepant finding may be partly accounted for by a difference in the sensitivity of the detection methods for the two substances. After administration of large doses of carotene, vitamin A and carotene were detected in the fetal liver, showing the permeability of carotene through the placenta, but since the detection was made only 24 hours after the administration, it is impossible to assert whether the detected vitamin A has been formed from carotene before or after the latter's transportation through the placenta. The yolk sac has been accepted as possessing secretory and absorbent function[11)12)13)], and the results of my experiments also suggest the possibility of its enjoying a function in absorbing and concerving fat and vitamin A, seeing that the sac was found to contain small quantities of fat and vitamin A, that these contents are depleted by long feeding with fat- and vitamin A–poor diet and that the contents thus depleted are recovered by administration of large doses of fat and vitamin A preparations. The increase of vitamin A in the syncytium cells after vitamin A administration and incubation vitamin A solution also suggests that the placenta has a function of storing vitamin A. As the mechanism for fat to pass through the placenta, we may either presume that fat is hydrolysed by lipase before the permeation, as in the small intestine and as advocated by Verzar[14)], or is let through as undecomposed fat, as advocated by Frazer[15)], but as in

Watanabe *et al*'s.[16] and Padykula's[17] as well as the present author's experiments, both lipase and fatty acids were found negative in the placenta, and the migrating fat in the blood vessels in the fetal side of the placenta of rats were found in form of small fat granules, the opinion that fat is decomposed by lipase before passing through the placenta must be denied. After incubation, both fat and vitamin A appeared as absorbed on the surface of the syncytium cells. This finding recalls the concentration of these substances on the microvilli, as Yamaguchi *et al.*[18][19][20][21] have observed under an electron-microscope, resulting in a concentration gradient in the vicinity and a transition of these substances into the syncytium cells. Upon addition of bovine bile to fat to disperse it into emulsion of fine granules, fat was found to permeate into the chorionic villi — another finding supporting the theory of Frazer. Yamaguchi *et al.*[18][19] opine that the Langhans cells have no part in the process of transition of substances through the placenta, and my experiments with vitamin A showed results endorsing this opinion. The basement membrane is known to be of so high electron density electron-microscopically and so thick that even Ca,[22] Cu,[23] Fe,[24] glucose[25] and such substances with small molecular weight are for a while detained in it. So, it seems quite natural that fat and vitamin A of large molecular weight should be impeded in their migration. Therefore, the quantity of fat and vitamin A transpiring into the stroma is only small and no such transportation through the lattice fibre ducts forming the extravascular transport system of fluid of Kihara[26], as found by Yamaguchi *et al*, with Ca etc. could be observed in the case with fat and vitamin A. It has been long accepted that the transformation of carotene into vitamin A is effected only in the liver[27], but more recently, the intestinal tract has come into attention as an organ where such a transformation takes place[28][29]. The present author also found such a transformation taking place in the intestine but not in the placenta and it is probable that both vitamin A as well as carotene pass through the chorionic epithelium in their original form unchanged.

SUMMARY

1) After administration of large quantities of fat and vitamin A to rats in the last stage of gestation, transition of these substances to the fetal side of the placenta was observed.

2) Fat does not pass through the placenta after its decomposition by lipase but in the original form of fat in fine granules.

3) Vitamin A is kept in reserve in the placenta and apparently also in the yolk sac.

4) The Langhans cells in the chorionic epithelium does not play a part in the transition of fat and vitamin A through the placenta. These substances pass through the interspaces between these cells.

5) Both fat and vitamin A whether administered to the maternal or the fetal side, were found detained in the basal layer.

6) Only small quantities of fat and vitamin A pass over into the chorionic stroma and a transport route via the extra-vascular aqueducts could not be established.

7) No intraplacental transformation of carotene into vitamin A could be observed.

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Studies on the Role of Noradrenaline in the Pathogenesis of Hypertension

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INTRODUCTION

Noradrenaline is one of the powerful pressor substances which are found physiologically in the human body. Noradrenaline produces a type of hypertension that closely resembles essential hypertension, when it is infused in man. During the infusion, elevation in both the systolic and the diastolic pressure occurs and this pressor effect is proportionate to the quantity of administered noradrenaline. But no significant subjective symptoms are observed in the range of dosage between 0.01 and 0.3 μg/kg/min.

On the other hand, in the cases of pheochromocytoma, hypertension is caused by a large quantity of noradrenaline released from the chromaffin cell tumor. Unfortunately, in about two-thirds of such cases the tumor induces sustained hypertension, so that the differential diagnosis between essential hypertension and pheochromocytoma is made very difficult.

Therefore, it is a very interesting problem to evaluate the role of noradrenaline in the pathogenesis of essential hypertension. The present study was undertaken for these reasons.

EXPERIMENTS AND RESULTS

1) Blood-pressure response to the infusion of noradrenaline.

According to Goldenberg's[1] technique, the noradrenaline sensitivity tests were performed in 8 normotensives, 10 patients with uncomplicated essential hypertension and one patient with surgically verified pheochromocytoma.

The results are shown in Fig. 1, in which the dosage is plotted as abscissa and systolic blood pressure as ordinate, so the steeper the curve, the higher the sensitivity. It is obvious that the hypertensives are more sensitive to noradrenaline than normotensive subjects. These experimental data are similar to Goldenberg's[1] results. On the other hand, in the case of pheochromocytoma

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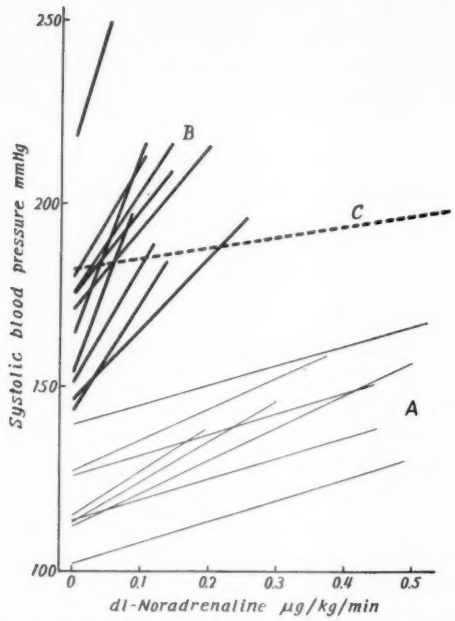

Fig. 1. The systolic-pressure responses to increasing doses of noradrenaline. A. normotensive group; B. hypertensive group; C. pheochromocytoma.

this test showed lower sensitivity than in normotensives. This seems to indicate that the high level of circulating pressor amine may actually obtund the responsiveness of the peripheral vascular system. If essential hypertension is caused by an excess of noradrenaline secretion, sensitivity to noradrenaline should be weakened as in this case of pheochromocytoma, but reverse results were obtained. Thus, our data suggest the genesis of essential hypertension is not due to an excess of noradrenaline released from sympathetic nerve endings or the adrenal medulla.

2) Blood-pressure response to Regitine.

Regitine (5 mg.) was administered intravenously to 68 cases with hypertension, and the relation between the change in blood pressure by this agent and the blood pressure before its injection was analysed. In this series, it was found that 2 cases of them had pheochromocytoma. Figure 2 demonstrates the typical response to Regitine in these two cases. Of the remaining 66 cases, 6 had renal hypertension, 3 Conn's syndrome and 57 essential hypertension.

Generally speaking, there was no relation between the response to Regitine

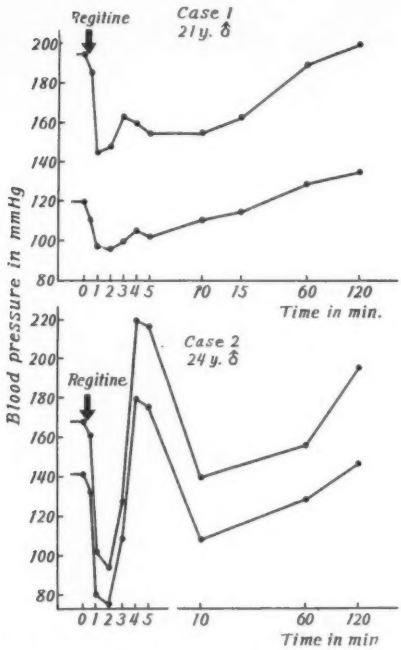

Fig. 2. Changes in blood pressure resulting from intravenous administration of 5 mg. of Regitine.

and blood pressure as shown in Figure 3.

Pharmacologically, Regitine possesses a marked adrenolytic effect on circulating pressor amine, while its sympathicolytic action is only very weak[2]. But the pressor effect of noradrenaline released from sympathetic nerve endings may be at least partially blocked by Regitine. Therefore, if hypertension is caused by an excess of noradrenaline, it may be expected that there is a parallel relation between the response to Regitine and the blood pressure.

Although it is questionable whether the response to Regitine may be taken as an indicator of noradrenaline production, the above results might indicate the cause of essential hypertension is not due to hypersecretion of noradrenaline.

3) Quantity of noradrenaline in plasma and urine.

a) Noradrenaline in plasma.

In order to evaluate the relation between hypertension and noradrenaline, venous blood plasma of 20 normotensives and 20 hypertensives were analysed for its content of noradrenaline according to Weil-Malherbe and Bone's[3] method.

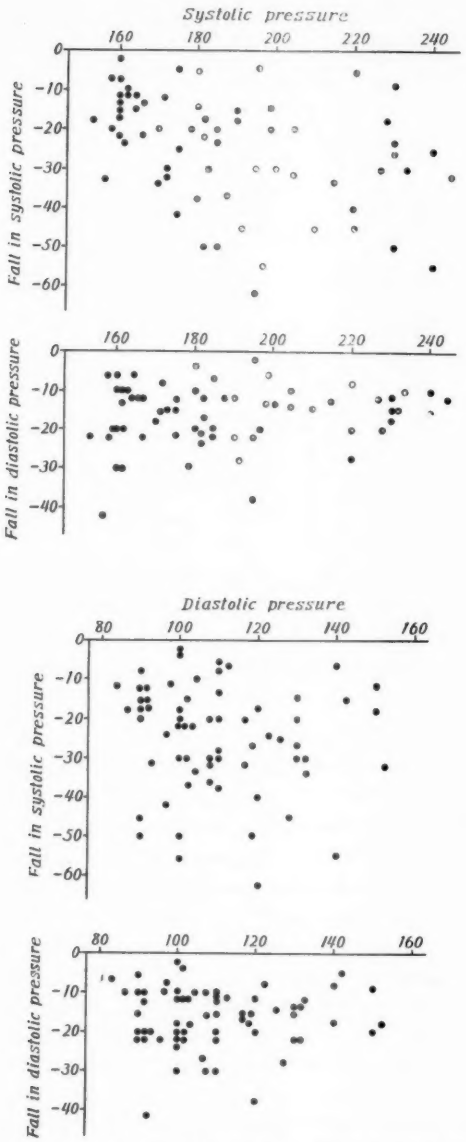

Fig. 3. The correlation between blood pressure and its response to Regitine.

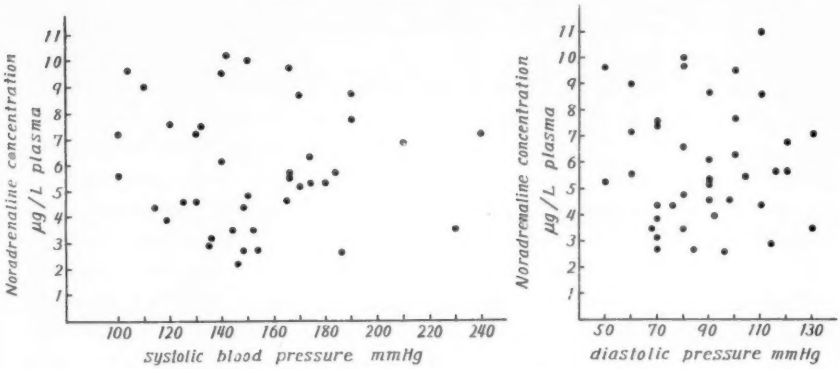

Fig. 4. Correlation diagram between blood pressure and noradrenaline concentration in plasma.

Blood samples were collected from the antecubital vein of patients lying quietly in bed at 10 a.m. Immediately before sampling, the blood pressure was measured. As shown in Figure 4, there was no parallelism between noradrenaline concentration and blood pressure.

For the study of the role of noradrenaline in hypertension, it is the most reliable and necessary way to estimate accurately the concentrations of noradrenaline in plasma. However, as we[4] have reported previously, the method used here is not specific enough for catecholamines. The trihydroxyindole method has a very high specificity, but it can not be used in determining the contents of catecholamines in small amount of plasma, because the concentrations of these amines in venous plasma are extremely low.

b) Noradrenaline in urine.

Urinary noradrenaline was determined using the trihydroxyindole method described by Euler and Floding[5]. The subjects consisted of 20 normotensives and 20 hypertensives. They were asked to void at 2 p.m., then to lie quietly in bed for an hour, and to void again at 3 p.m. Their blood pressure was registered at 2.30 p.m. Thus the urine excreted during one hour was collected excluding the postural influence on noradrenaline excretion.

The results are shown in Figure 5. There was a tendency for the urinary noradrenaline to decrease with the rise of the blood pressure levels.

The technique used here is highly specific and its results were reported to be in good agreement with that of bioassay.

The amount of noradrenaline recovered in urine was reported[6] to amount to only 2–3 per cent of the infused quantity, but this rate is similar in various conditions except in advanced renal damage. Therefore, the urinary noradrenaline can be considered as a good indicator of its endogenous production. The

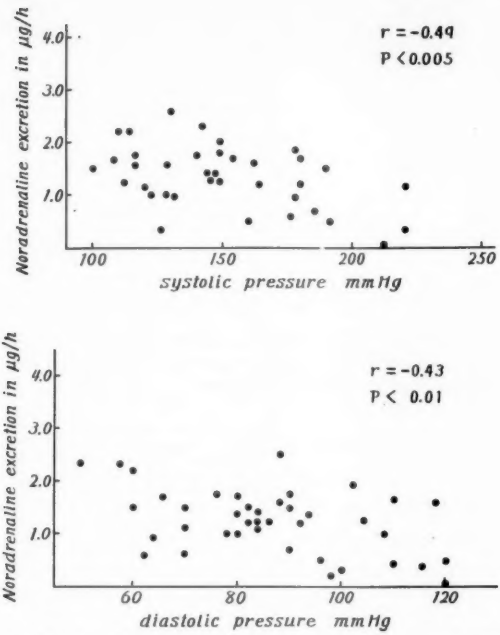

Fig. 5. The correlation between blood pressure and urinary noradrenaline at rest.

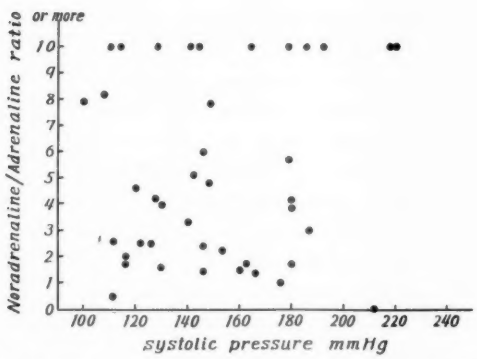

Fig. 6. The correlation between systolic blood pressure and noradrenaline/adrenaline ratio.

above results also indicate that the cause of essential hypertension is not the excess of this pressor amine.

The ratio noradrenaline/adrenaline was calculated from the above mentioned data, and it was plotted against the systolic blood pressure (Figure 6). No correlation was noted between them.

However, since the standard deviation of repeated estimations of these amines was about $\pm 10\%$ in our laboratory, and since a few per cent of noradrenaline was occasionally estimated as adrenaline and *vice versa*, the noradrenaline/adrenaline ratio is not so exact that any decisive conclusions could be derived from this calculated ratio. But the results described above do not support the Goldenberg's hypothesis[11], that the increase of noradrenaline/adrenaline ratio is an important genetic factor in essential hypertension.

DISCUSSION

Many authors have investigated the role of noradrenaline in the pathogenesis of essential hypertention. The urinary excretion of noradrenaline in hypertensives was studied by Euler[7], Burn[8], Goldenberg[9] and so on, but they all found no difference between hypertensives and normotensives in this respect.

Sundin[10] found that the hypertensive patients excrete about the same amount of noradrenaline as the normotensives, but when the former are tilted to 75° no increase takes place in noradrenaline excretion, while marked increase is regularly observed in the latter in the same condition. Recently Mϕller[11] reported that the amounts of urinary noradrenaline decrease with the rise of blood pressure levels.

Both Sundin and Mϕller concluded that the activity of the sympathetic nervous system of hypertensives is suppressed reflexly to adapt to their internal environment, resulting in the decrease of noradrenaline production at nerve endings. Our results support their view.

Hypertensives react more markedly to infused noradrenaline than normotensives. Not only noradrenaline but many other pressor substances are known to cause higher response in the former than in the latter. What role does this hyperresponsiveness play in the genesis of hypertension? And what is the mechanism that produces this hyperresponsiveness? Freedman[12] and Tobian[13] extensively investigated these problems, but they have not succeeded in arriving at a satisfactory answer.

It is desired these problems should be studied more profoundly in the future.

SUMMARY

The role of noradrenaline in essential hypertension was studied and the following results were obtained.

(1) Hypertensive subjects except the patient with pheochromocytoma

were found more sensitive to the infusion of noradrenaline than normotensives. But in the cases with pheochromocytoma the sensitivity was weaker than in normotensives.

(2) There was no relation between the blood pressure and its response to Regitine.

(3) A negative correlation was observed between blood pressure and urinary excretion of noradrenaline.

From these experiments it may be concluded that hypertension (except in the case of pheochromocytoma) does not result from the excess of noradrenaline. Rather, in hypertensive subjects, it seems to be likely that the secretion of noradrenaline is reflexly decreased in order to adapt to their hypertensive state. On the other hand, the sensitivity to noradrenaline is obviously excessive in hypertensives. But the cause and the role of this hypersensitivity are entirely unknown to date.

These problems await future study.

ACKNOWLEDGEMENT

We are indebted to Prof. Dr. T. Torikai for his continuous leadership throughout these studies.

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